THE CHEMISTRY of NANOMATERIAL and NANOBIOMATERIAL

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ABSTRACT

Nanomaterials and nanobiomaterials are materials which are currently subject to intense research while at the same time their usage in development of various technological applications is also moving at a very swift pace. Advances in this area of research are consequent to the realization that nanomaterials and nanobiomaterials have profound influence on improvement of various aspects of life quality as well as other industrial aspects. Layered two dimensional inorganic materials, especially layered double hydroxides (LDH) have versatile properties due to its intercalation and deintercalation characters. By hybridization of LDH with beneficial functional active agents, various new host-guest nanohybrids with tailor made properties can be generated. If the guest is a biomaterial such as DNA or plasmid, then the nanohybrid can be used as vector for plasmid transfaction. On the other hand, if a drug is encapsulated into the LDH interlamellae, then the nanohybrid can be used as a controlled release formulation. On the other hand, if active agents such as herbicides, plant growth regulators, etc. are used, then a new generation of agrochemicals which is safer, user- and environmentally-friendly can be generated. LDH composed of nickel calcined at high temperature of around 800 ^{o}C is suitable to be used as the catalyst precursor for carbon nanotubes preparation. This is due to the better dispersion of the nickel nanoparticles used for the formation of multiwalled carbon nanotubes. This work provides understanding of some important fundamental considerations necessary for the design and formation of new functional materials especially nanomaterials and nanobiomaterials of nanohybrid type. The materials can be used to store beneficial agents in the nanolayered interlamellae, transport it to the target and subsequent controlled release. In addition, using biomaterial such as bacteria as biotemplating agents coupled with

the hydrothermal method to generate new nanostructured materials open up new methods of synthesis by the green chemistry route. These offer avenues for a new class of nanomaterials with physical and chemical properties that can be tailor made.

Keywords: Nanomaterial, Nanobiomaterial, Carbon nanotubes, Controlled release formulation, Layered double hydroxide, Hydrotalcite, Plasmid.

INTRODUCTION

Nanoscience and nanotechnologies are the area of studies concerned with producing new or enhanced materials at nanoscale regime. A nanometer, nm, is one thousand millionth of a meter, i.e. $1x10^{-9}$ m. For example, a red blood cell is approximately 7,000 nm wide and a water molecule is around 0.3 nm across.

Nanomaterials can be constructed by "top down" or "bottom up" techniques. In the former process, nanostructures are generated from larger pieces of materials, for example by etching, while in the latter technique, the structures are constructed atom by atom or molecule by molecule, whereby the atoms or molecules arrange themselves into a structure and the structure is governed by physicochemical properties of the atoms or the molecules.

Intense research interest in nanomaterials and nanobiomaterials, with scales from 100 nm down to 0.2 nm (the size of atom) is due to the fact that at this scale regime, the properties of materials can be very different from those of a larger size. This is because the bulk properties of materials often change dramatically at nano size. At this scale, materials have a relatively larger surface area compared to the same mass of materials in a larger form and therefore the materials are chemically more reactive, which affects their strength or electrical properties. In addition, quantum size effects dominate the behavior of matter at the nanoscale thus affecting the optical, electrical and magnetic properties (Drachev *et al.,* 2004).

Nanotechnology and Nanobiotechnology

Nanotechnologies are defined as the design, characterization, production and application of structures, devices and systems by controlling shape and size at nanometer scale (Dowling, 2004). On the other hand, biotechnology is field of biological sciences directed at manipulation of biologics to achieve an improved state

and objective. The design and manipulation of nanomaterials for biotechnological processes at the nanometer-scale is called nanobiotechnology.

Green plants convert more energy and synthesize greater tonnage of fine chemicals than the entire combined global chemical industry. This is an example of amazing nanobiotechnology process (Drexler, 1999) to meet human needs. Such a process is also a basic process for life, right at the beginning of cell division when the zygote is formed to the end, when the body decomposes. Other complex nanobiotechnology processes include energy conversion and transduction, synthesis and function of very specific fine chemicals such as hormones, enzymes, information storage in neuron and neurotransmitter, senses, signal, movement, to name a few. A ribosome is capable of self-assembly from more than 50 different proteins, eukaryotic cells direct DNA synthesis, with an error rate of less than 10^{-11} per nucleoutide (Lowe, 2000).

High performance bones and teeth in human are as a result of smart and complex bionanotechnology processes. Complex architecture of dentin with hydroxyapatite honeycomb structure, (Figure 1a) make it stronger than its counterparts (Risnes, 1998). Similar smart architecture and design is seen in the abalone shell structure which is composed of layered protein and aragonite (calcium carbonate) (Figure 1b), and other creatures created in order to meet the functions specified for them (Lin and Meyers 2005). Its architecture and design is the key to why the abalone shell is so tough. It is the architecture and design which makes the shell almost 3000 times stronger than the materials it is made out of alone i.e. calcium carbonate and protein.

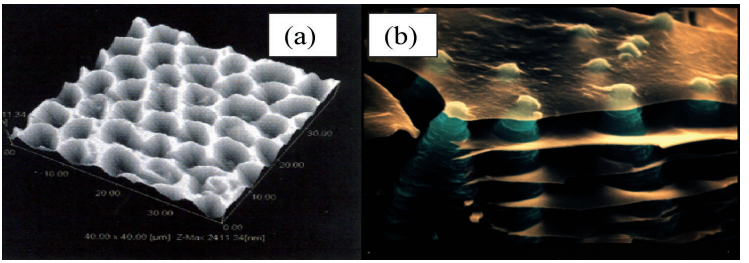


Figure 1 AFM image of teeth after etching process showing honeycomb-like structure of apatite (a) and structure of abalone shell composed of calcium carbonate-polymer layered structure (b).

Chemists have been making polymers through self-assembly nanotechnology, in which nanoscale subunits, monomer is bonded together to form strong long chain macromolecules. Similarly, for many decades, zeolites, a nanoporous material was synthesized and widely used as catalysts for various industrial processes, especially for downstream petroleum industries which have a high impact to our daily life.

Lately, layered inorganic materials have been the subject of intense research due to their wide technological applications (Cavani *et al.,* 1991, Komarneni *et al.,* 1995, Choy *et al.,* 2004, Gunawan and Xu, 2008).

LAYERED INORGANIC MATERIALS

Layered material is a two-dimensional (2D) material generally composed of inorganic layers separated by interlayers containing ions. If anion is the species which constitutes the interlayers, the resulting material is called anionic clay. On the other hand, if cation occupies the interlayers, then it is called cationic clay. Montmorillonite and kaolinite, are some examples of the former and hydrotalcites or layered double hydroxides are examples of the latter.

Layered Double Hydroxides

Hydrotalcites with inorganic layers composed of cations other than Mg and Al, and anions other than carbonate is called hydrotalcite-like or layered double hydroxide (LDH). The general formula for LDH is $[M_{1-x}^{2+}M_x^{3+}(OH)_2]^{x+}A_{x/n}^{n-}.yH_2O$, where M^{2+} are divalent cations, M^{3+} are trivalent cations, A^{n-} is the interlayer anion and x is the mole fraction of $M^{3+}/(M^{2+}+M^{3+})$ having a typical value of 0.25 (Cavani *et al.,* 1991).

Structurally, LDHs are formed by brucite-like $[Mg(OH)_2]_n$ sheets where isomorphous substitution of Mg^{2+} by a trivalent cation like Al^{3+} occurs. The positive charge of the layer is compensated by counter anions, to render the resulting material charge neutral. These anions occupy the interlayer space along with water molecules, as shown in Figure 2.

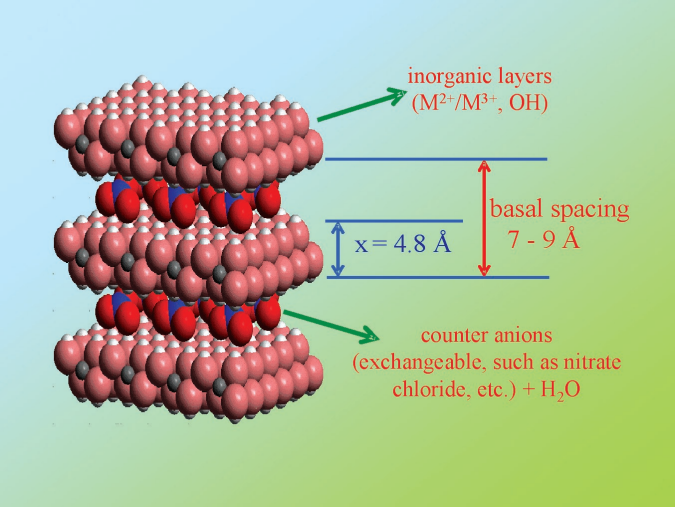


Figure 2 Structure of LDH showing the inorganic layers composed of M^{2+}/M^{3+} cations and exchangeable counter anions to render the resulting material charge neutral. H_2O is also present in the interlayers**.**

The basal spacing of LDH is around 7 - 9 Å, depending on the type of counter anion and the amount of water present. The thickness of the inorganic layer is 4.8 Å, which means that the space occupied by the counter anion is around 2.2 - 4.2 Å.

The counter anions are bonded to the inorganic layer sheets by electrostatic interaction or ionic bond. They are exchangeable, if anionic species of higher affinity towards inorganic LDH sheets are present, and they act as the driving force to enable the ion exchange process to take place. Such a phenomenon can be exploited for the formation of host-guest type of material or nanohybrids, as well as controlled release formulation.

Zinc Layered Hydroxide

Layered zinc hydroxide (ZLH) has similar structure to that of LDH. The difference is that the inorganic brucite-like sheets are composed of only one metal cation, Zn^{2+}, instead of two as in the LDH materials.

With 2D layered structures similar to LDH, ZLH can also be used as a host for host-guest type materials. If organic or bioorganic anion is encapsulated into the interlamellae, then new nanohybrid compounds can also be formed.

NANOLAYERED HYBRID MATERIAL

Hybridization of beneficial organic guest into inorganic host affords scientists an opportunity to synthesize various combinations of new organic-inorganic nanohybrids with various potential applications, especially for controlled delivery of beneficial agent and storage (Tyner *et al.,* 2004, Xu and Lu, 2006).

If the anion of the 2D layered materials such as LDH of ZLH is replaced by other anions, formation of the so-called host-

guest nanolayered materials can be generated. For this type of material, the LDH or ZLH can be regarded as host and the anions, especially active agents or beneficial agents such as drugs (Xu *et al.,* 2006), gene and pharmaceutical products (Kriven *et al.,* 2004), agrochemicals such as plant growth regulators (Hussein *et al.,* 2002a,b), herbicides such as 2,4-dichlorophenoxyacetate (Hussein *et al.,* 2002c) and dyes (Hussein *et al.,* 2002d, 2004a,b) as guests. Such materials are also called nanohybrids.

Basically anions of any type, especially active and beneficial agents of organic, polymer, inorganic or biomaterials can be hybridized into the LDH interlayers. However, parameters such as pH, aging time and the ratio of the anion are important as it controls the physicochemical properties of the resulting synthesized materials so that they are phase pure and well crystallized.

Due to the bulky size and/or spatial orientation of the guest anions, the resulting interlamellae have to be expanded to accommodate the anions. As a result, the basal spacing shifts from Angstrom to nanometer size, hence the term "nanohybrid". This is shown in Figure 3.

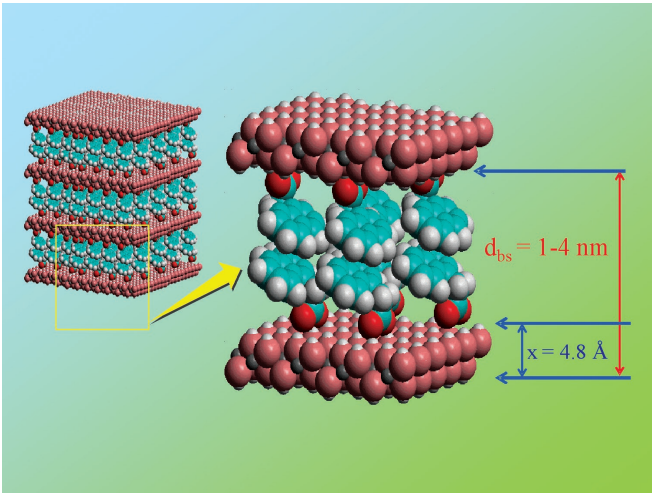


Figure 3 Structure of layered organic-inorganic nanohybrid showing expanded inorganic LDH sheets to accommodate the bulky and spatial orientation of the guest molecule at nanometer size level.

Nanohybrid for Active Agent Delivery with Controlled Release Capability

Delivery of beneficial agents through controlled release formulation (CRF), such as drugs in humans and active agents in environmental and industrial applications, have of late attracted increasing interest.

The sustained release property of host-guest nanolayered type of materials or nanohybrids can be exploited as a controlled release formulation. This is due to the intercalation and deintercalation processes that are reversible, and the memory effect property of the LDH (Figure 4).

Drug delivery especially through CRF has advantages compared to its counterparts, such as drug pharmacokinetics, as it can be tailor made, maintain pharmacologically active drug levels for long

periods – avoiding repeated administrations, act as a vector of drug to the pharmaceutical target and increase the solubilization rate and bioavailability of drugs, minimize adverse reactions or maximize efficacy with tailor made properties and promote higher stability of the active agents in the formulation.

The interactions between the matrix and the active substances are important and this in turn may control the controlled release properties of the formulation such as diffusion, reaction rates or other physicochemical parameters. In addition, manipulation of the appropriate barriers, the target and the fate of the active agent once beyond these barriers are equally important and contribute to the success of the controlled release system designed.

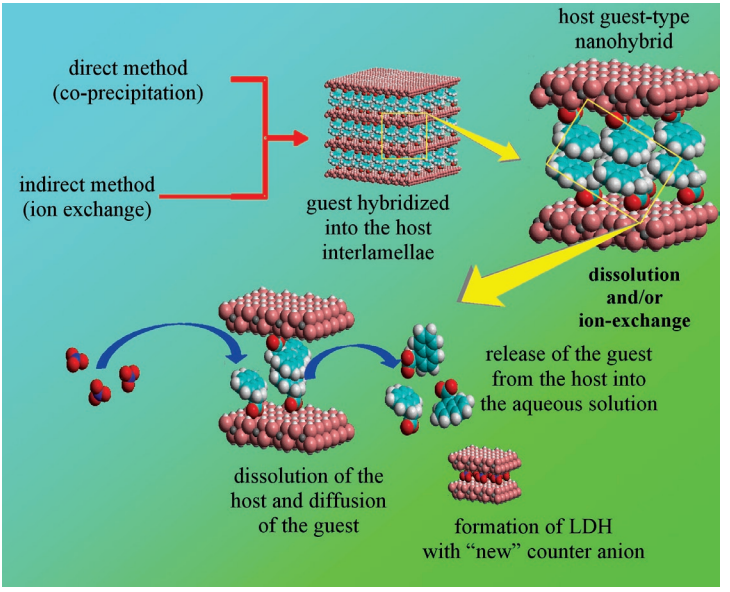


Figure 4 Hybridization of beneficial agents into LDH for the formation of layered organic-inorganic nanohybrid, and controlled release process of the intercalated agent by dissolution and diffusion processes. Memory effect property yielded LDH with new counter anion.

Figure 4 shows schematically the hybridization of beneficial agent into LDH for formation of layered organic-inorganic nanohybrid. Direct co-precipitation and indirect ion exchange methods are the two most common techniques used for the hybridization process. As a result, the anionic species or the guest will be encapsulated into the LDH and the interaction between the host and the guest is due to ionic bond, a strong bond that results in a very stable nanohybrid compound.

When the nanohybrid is contacted with other anions, especially those with higher affinity towards the inorganic LDH sheets than the anion that was already intercalated, ion exchange process will take place. As a result, the intercalated species (guest anion) will be released or deintercalated. At the same time dissolution of the host and diffusion of the guest take place, resulting in a sustained or controlled release of the guest. Due to the "memory effect" property, a "new" LDH with new counter anion, an anion that is present in the contact solution, will be formed.

PHENOXYHERBECIDE NANOHYBRIDS

Synthesis and Physicochemical Properties

Phenoxyherbecides, namely 2-(2,4-dichlorophenoxy)butyric acid (DPBA), 2-(2,4-dichlorophenoxy)propanoic acid (DPPA) and 2-(3-chlorophenoxy)propanoic acid (CPPA) are the most widely used agrochemical pesticides. Through optimization of important synthesise parameters, intercalated organic-inorganic of the 3 organic moieties to form 3 different new nanohybrids were accomplished. The nanohybrids were used as controlled release formulations and by virtue of the ion exchange property, the organic moiety was subsequently controlled released.

Pure phase, well ordered nanohybrids were synthesised by co-precipitation method. The resulting nanohybrids obtained by hybridization of LDH with CPPA, DPPA and DPBA, are labelled as CPPADI, DPPADI and DPBADI, respectively. PXRD patterns (Figure 5) show that the expansion of the inorganic LDH interlayers occured from 8.90 Å in LDH to 20.02 - 28.14 Å in the nanohybrids. The expansion is to accommodate the bulky phenoxyherbicides molecules, together with their spatial orientation in the interlayers.

PXRD patterns of the nanohybrids show very ordered nanolayered structures for all the DPBADI, DPPADI and CPPADI with 8, 4, 5 harmonics, respectively. By using these reflection values together with Bragg's equation, the basal spacing was deduced, as given in Table 1. This is the observed basal spacing of the nanohybrids.

Table 1 Basal spacing and chemical composition of LDH, DPBADI, DPPADI and CPPADI nanohybrids.

Sample	basal spacing (d)/Å	Zn/Al molar ratio	molecular fraction (X_{Zn})	C/ (N) (%)	[a]loading percent-age of the anion (% w/w)	molecular weight of the anion (g/mole)
LDH	8.90	2.8	0.74	(3.0)	-	-
DPBADI	28.14	2.0	0.67	26.4	54.72	249.09
DPPADI	21.44	3.0	0.75	21.6	47.77	235.07
CPPADI	20.02	2.9	0.74	20.6	38.26	200.62

[a] = estimated from CHNS analysis based on pure DPBA, DPPA and CPPA.

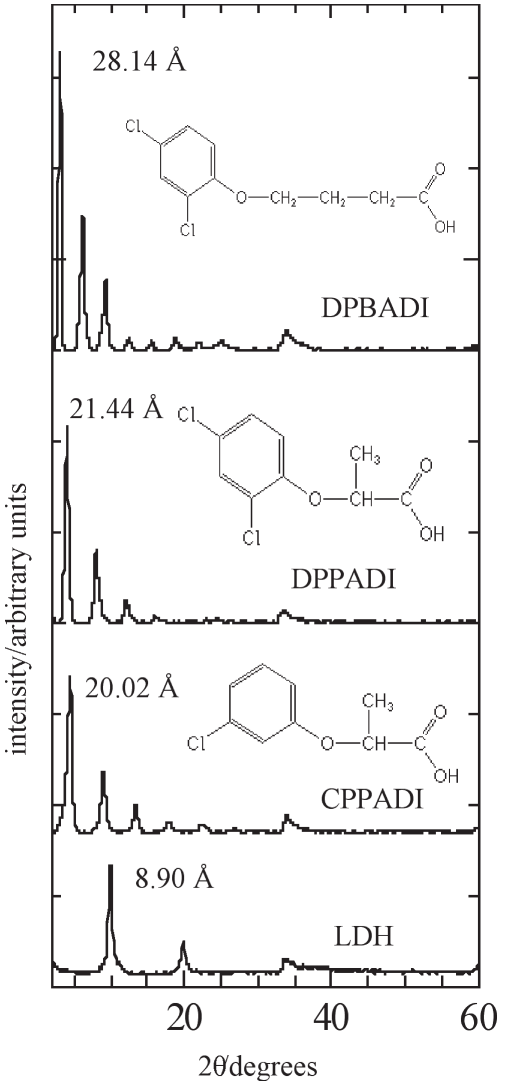


Figure 5 PXRD patterns of LDH and its nanohybrids, DPBADI, DPPADI and CDPADI. The molecular structure of the guest is given in the inset.

Basal Spacing and Spatial Orientation of the Guest Molecule

In order to get some insight into the spatial orientation of the molecules inside the inorganic interlamellae, the size of each of the molecules was calculated, as shown in Figure 6 and the dimensions are given in Table 2. It is known that the thickness of inorganic LDH is 4.8 Å (Figure 2), therefore the calculated geometry + 4.8 Å, is the expected basal spacing value of the guest molecules with the spatial orientation either to x, y or z axis inside the inorganic interlayers of the nanohybrids.

Comparison of the calculated and observed basal spacing values of the nanohybrids (Table 2) shows that the DPBA molecule is in the x axis orientation with respect to the LDH inorganic interlayers in the nanohybrid, DPBADI compared to y orientation for both DPPA and CPPA in their nanohybrids, DPPADI and CPPADI, respectively.

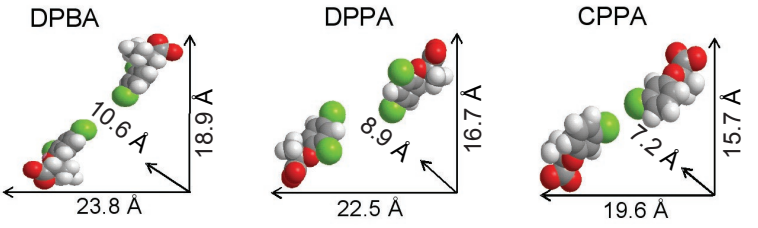


Figure 6 The calculated dimensions of DPBA, DPPA and CPPA.

Table 2 Observed and calculated values of the basal spacing of the nanohybrids. The calculated theoretical volume of the organic moiety is also given.

Nanohybrid	observed d (Å)	calculated basal spacing (calculated geometry + 4.8 Å)			calculated geometry (Å)			volume (Å^3)
		x	y	z	x	y	z	
DPBADI	28.14 (8)[a]	28.6	23.7	15.4	23.8	18.9	10.6	4768
DPPADI	21.44 (4)[a]	27.3	21.5	13.7	22.5	16.7	8.9	3344
CPPADI	20.02 (5)[a]	24.4	20.5	12.0	19.6	15.7	7.2	2215

[a](number of values fitted to Bragg's equation to calculate the d values)

Controlled Release Property

Release profiles of the anions from its nanohybrid interlayers into various concentrations of Na_3PO_4 solutions were studied at 0.008 – 0.001 M (Figure 7). The accumulated amount of the herbicide anions released from its nanohybrid increased as the concentration of sodium phosphate solution increased (Table 3).

Table 3 Accumulated amount of herbicides released from their nanohybrids at various concentrations of sodium phosphate solution

	release/%		
Na_3PO_4/M	**DPBADI**	**DPPADI**	**CPPADI**
0.001	31	39	35
0.002	42	45	74
0.005	44	65	86
0.008	56	70	92

Kinetic Release

Kinetic release of DPBA, DPPA and CPPA from their respective nanohybrids was analyzed using four kinetic models, zeroth order (Valelas *et al.,* 1995), first order, pseudo-second order (Lu *et al.,* 2006) and parabolic diffusion (Kodama *et al.,* 2001). The equation is given as in Equation 1, 2, 3, and 4, respectively.

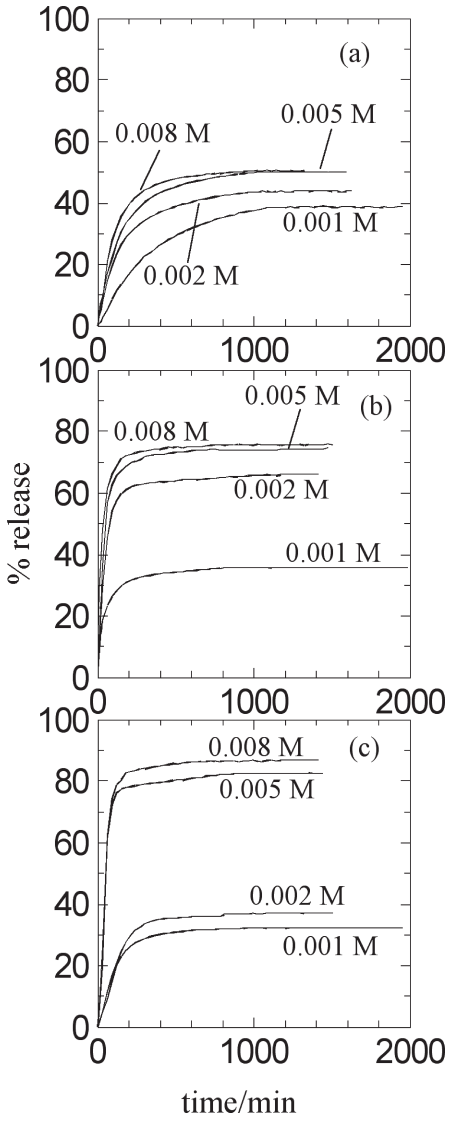


Figure 7 Release profiles of (a) DPBA, (b) DPPA and (c) CPPA from the interlamellae of their nanohybrids into the aqueous solutions containing various concentrations of Na_3PO_4.

$$x = t + C \quad (1)$$

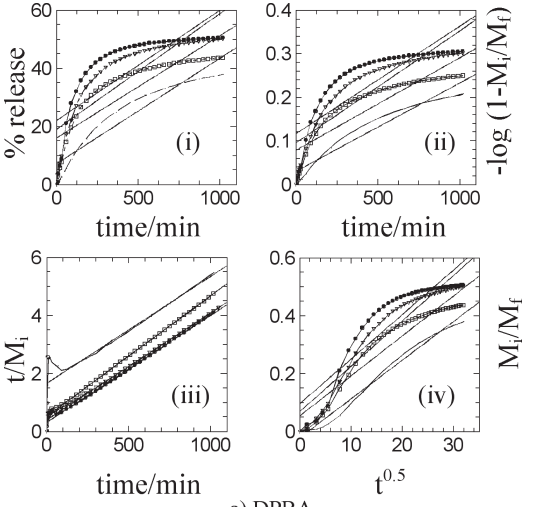
$$-\log (1-M_i/M_f) = t + C \quad (2)$$

$$t/M_i = 1/kM_f^2 + t/M_f \quad (3)$$

$$M_i/M_f = kt^{0.5} + C \quad (4)$$

where x is the percentage release of herbicides anion at time t, M_i and M_f are the initial and final concentrations of herbicides anions, respectively and C is a constant.

Figures 8 (a-c) show the fitting of the release amount of DPBA, DPPA and CPPA from their respective nanohybrids into sodium phosphate solutions at various concentrations for 0-1000 minutes to zeroth-, first- and pseudo-second order kinetics as well as parabolic equations.



a) DPBA

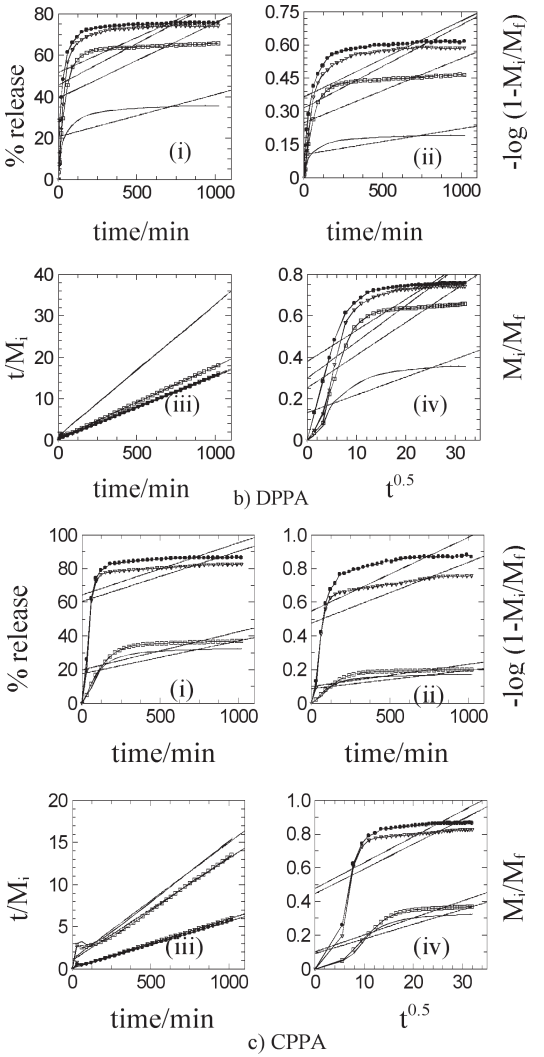


Figure 8 Fitting of the data for the release amount of the guest into the aqueous solutions containing various concentrations of Na_3PO_4; 0.001 M (circles), 0.002 M (squares), 0.005 M (triangles) and 0.008 M (dot) to the zeroth (i), first (ii) and pseudo-second order (iii) kinetics, and parabolic diffusion (iv) for t = 0-1000 min, DPBA (a), DPPA (b) and CPPA (c).

As a result of the fitting, the parameters, namely correlation coefficients, r^2 , and rate constant, k ,were obtained as summarized in Table 4. In addition, $t_{1/2}$ values i.e. the time required for 50 % of DPBA, DPPA or CPPA to be released from the nanohybrids, are also given in the table.

As shown in Table 4 and Figures 8a-8c, the correlation coefficients (r^2) for the pseudo-second order is fitted better than the first order, zeroth order and parabolic diffusion models for all the nanohybrids. This indicates that the pseudo-second order is better, able to describe the release kinetics of DPBA, DPPA and CPPA from their DPBADI, DPPADI and CPPADI nanohybrids, respectively under our experimental conditions. This shows that the release of the organic moiety from the inorganic LDH interlayer involved dissolution of the nanohybrid as well as ion exchange between the intercalated anions in the inorganic interlayer of LDH and the phosphate anions in the aqueous solution. The release process is governed by pseudo-second order kinetics.

In order to compare the release behaviour of DPBA, DPPA and CPPA from DPBADI, DPPADI and CPPADI nanohybrids respectively, the cumulative percentages released were used (Table 3 and Figure 9). For all the nanohybrids, the cumulative percentage release increases as the concentration of the sodium phosphate increases. This is obvious because as the concentration of the phosphate increased, more phosphate anions are available to be ion exchanged with DPBA, DPPA or CPPA, resulting in higher cumulative percentage release.

Table 4 Rate constants, $t_{1/2}$ and correlation coefficients obtained from fitting of the release amount of DPBA, DPPA and CPPA from their nanohybrids into various concentrations of Na_3PO_4 solution.

Na_3PO_4 concentration (mol L^{-1})	zeroth order	first order	parabolic diffusion	pseudo-second order		
	r^2			r^2	k (x 10^{-3})	$t_{1/2}$ (min)
DPBADI						
0.008	0.893	0.927	0.984	0.910	0.042	94
0.005	0.726	0.783	0.909	0.995	0.033	119
0.002	0.715	0.780	0.902	0.996	0.033	138
0.001	0.621	0.648	0.833	0.993	0.008	644
DPPADI						
0.008	0.505	0.546	0.734	1.000	0.044	31
0.005	0.424	0.494	0.655	0.999	0.080	126
0.002	0.413	0.506	0.645	0.999	0.140	194
0.001	0.376	0.476	0.030	1.000	1.035	345
CPPADI						
0.008	0.552	0.588	0.765	0.990	0.292	19
0.005	0.552	0.590	0.765	0.989	0.237	25
0.002	0.290	0.439	0.492	0.998	0.138	95
0.001	0.291	0.454	0.501	0.999	0.159	96

Comparison of the $t_{1/2}$, k values and cumulative percentage release shows that the phenoxyherbecides, the guest, is easier to be released is in the order of,

CPPA > DPPA > DPBA

Similar order to the volume of the phenoxyherbecides (Table 2) shows that the bulky guest is less readily released. Therefore the size of the guest plays an important role in determining the

release kinetic. This shows that by using different concentrations of the incoming anion to be ion exchanged with, the size and type of phenoxyherbecides to be released, the kinetic release of the herbicides can be tuned and hence the release process of the herbicide can be tailor made.

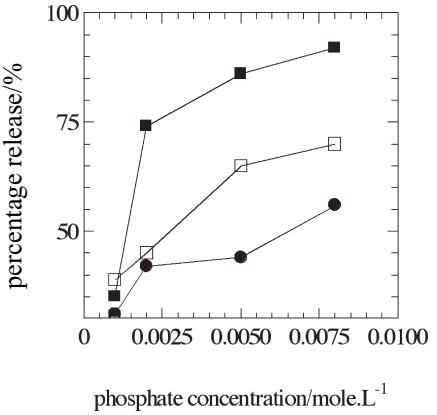


Figure 9 The effect of phosphate concentration on the cumulative percentage release of CPPA, DPPA, DPBA from their nanohybrids, DPBADI (●), DPPADI (□) and CPPADI (■), respectively.

Formation of Nanohybrid and Nanobiohybrid with LDHs using Various Guests with and without Controlled Release Study

Apart from phenoxyherbecides, various beneficial agents with specific functions were also studied for the formation of their LDH-nanohybrids and this is summarized in Table 5. Where appropriate, the controlled release property of the resulting nanohybrids was also studied.

Various synthesise parameters, such as pH, anionic and cationic ratios and methods of preparation were optimized and these parameters were found to be very much dependent on the

type of the hosts as well as the guest, for the formation of the nanohybrids. Similarly, the controlled release property of the resulting nanohybrids is also dependent on the crystallinity, method of synthesis, the nature of the incoming exchangeable anions and their concentration. All these properties can be exploited so that the release can be tuned and tailor made.

Table 5 Formation of nanohybrids and nanobiohybrids by hybridization of various guests with LDHs and its controlled release properties

Organic moiety/ biomaterial	**FON**	**CRF**	**Autors and year published**
Plasmid DNA	√	√	Masaruddin *et al.*, 2009
Gallate	√	√	Hussein *et al.*, 2009
Metanilate	√	√	Hussein *et al.*, 2008a
Glutamate	√	√	Md. Ajat *et al.,* 2008
4(2,4- Dichlorophenoxy) butyrate	√	√	Hashim *et al.*, 2007
4-Chlorophenoxyacetate	√	√	Hussein *et al.*, 2007
Pamoate	√	√	Jubri *et al.*, 2007 Husein *et al.*, 2004d
Evan's blue	√	–	Hussein *et al.*, 2004a
Acid Fuchsin	√	–	Husein *et al.*, 2004b
Indole-2-carboxylate	√	–	Hussein *et al.*, 2004c
Anthraquinone-2-sulphonate	√	–	Asmah *et al.*, 2003 Hussein *et al.*, 2002f
1-Naphthaleneacetate	√	√	Hussein *et al.*, 2002a,b

2,4-dichlorophenoxyacetate	√	√	Hussein *et al.*, 2002c
Naphthol blue black	√	–	Hussein *et al.*, 2002d
Dioctyl sulfosuccinate	√	–	Hussein *et al.*, 2002e Hussein *et al.*, 2000b
2-Acryamido-2-methylpropane sulfonate	√	–	Hussein *et al.*, 2001a
Anthraquinone-2,6-disulfonate	√	–	Hussein *et al.*, 2001b Hussein *et al.*, 2000a
Dodecyl sulphate	√	–	Hussein *et al.*, 2000c

[a]FON = formation of nanohybrids or nanobiohybrids, [b]CRF = controlled release property

NANOBIOHYBRID

Hybridization of biomaterials (as guest) into inorganic interlayers (as host) in LDH has many advantages such as increased stability of the guest compared to its counterpart and for use as a means for safe storage of unstable materials under extreme environments. For example, bare DNA usually has to be stored at low temperature compared to room temperature for DNA in nanobiohybrid form (Choy *et al*., 2000). In addition, the resulting nanobiohybrid can also be used as a nonviral vector for gene delivery if the gene is used as the guest and the release is of controlled manner (Masarudin *et al*., 2009).

Glutamate-Nanobiohybrid

Amino acid, glutamate, can be hybridized into LDH for the formation of glutamate-nanobiohybrid. Optimization of various synthesis parameters resulted in the formation of pure phase bio-iorganic nanohybrid (BINH) material. The glutamate is in

perpendicular orientation to the LDH inorganic interlayers in which the basal spacing of the host expanded from 8.9 in the LDH to 12.7 Å in the nanohybrid, to accommodate the amino acid (Figure 10) (Ajat *et al*., 2008).

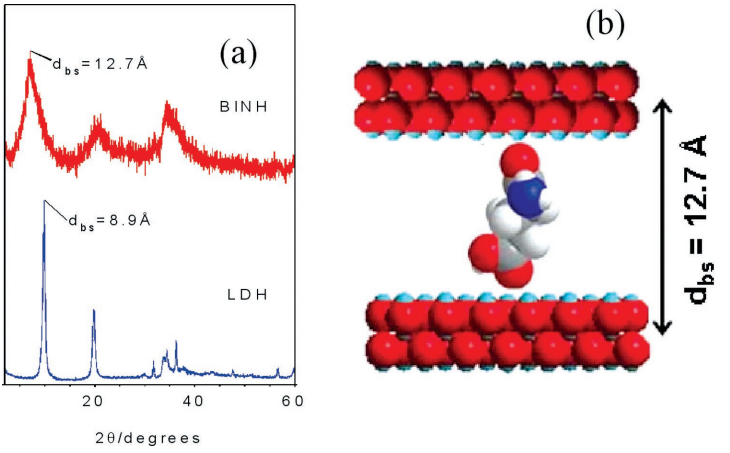


Figure 10 Formation of bio-inorganic nanohybrid (BINH) by intercalation of glutamate into the LDH inorganic interlayer with basal spacing expanded from 8.9 in the LDH to 12.7 Å in the BINH (a) and molecular calculation shows that the glutamate is in perpendicular orientation to the LDH inorganic interlayers (b) (Ajat *et al*., 2008).

Nanobiohybrid as Gene Delivery Vector

Formation of the LDH–DNA nanobiohybrid can be exploited as the delivery vector of DNA. Figure 11 shows schematically the formation of the LDH–DNA nanobiohybrid and delivery steps of the DNA into the nucleus for final expression (Choy *et al.,* 2000).

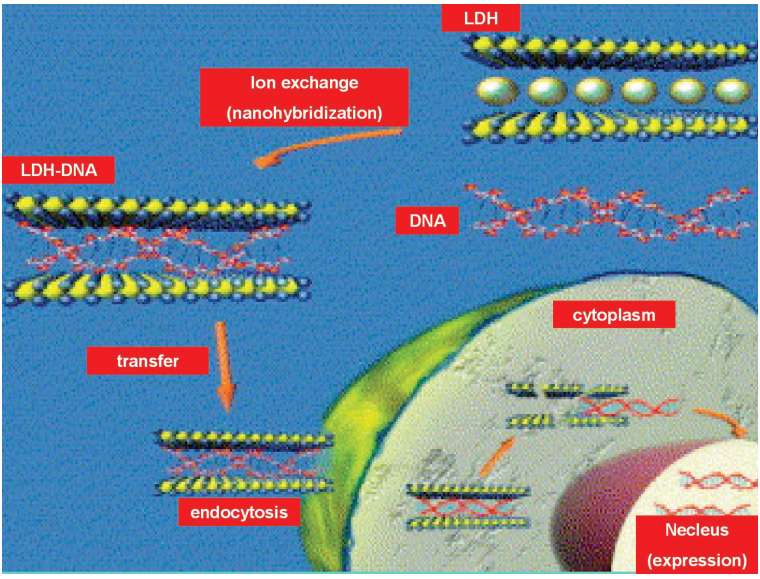


Figure 11 Schematic representation showing the formation of LDH–DNA nanobiohybrid and delivery steps of the DNA from the nanobiohybrid into the cell and finally into the nucleus for expression. The step involves the hybridization of DNA into the LDH for the formation of nanobiohybrid followed by transfer or endocytosis. Once in the cytoplasm, deintercalation of the DNA occurs and the DNA is subsequently transferred into the nucleus for expression (Choy *et al.,* 2000).

LDH and DNA can be hybridized to form nanobiohybrid for example by ion exchange or direct co-precipitation method. As a result of the intercalation process, the DNA will be encapsulated into the LDH interlayers and form a new compound due to the formation of a stable ionic bond between the LDH and the DNA.

The LDH-DNA nanobiohybrid can then be transferred into the cell (endocytosis) and finally into the nucleus for expression. Once in the cytoplasm, deintercalation of the DNA occurs and subsequently the DNA is transferred into the nucleus for expression.

Plasmid-Nanobiohybrid

The delivery of a full plasmid, encoding the green fluorescent protein gene into African monkey kidney (Vero3) cells, was successfully achieved using nanobiohybrids based on LDH (Masarudin *et al.,* 2009). This demonstrated the potential of using nanohybrids as an alternative DNA delivery vector. Intercalation of the circular plasmid DNA, pEGFP-N2 into Mg-Al-nitrate LDH was accomplished through anion exchange routes to form new nanobiohybrid material. The host was first synthesized at Mg^{2+} to Al^{3+} initial molar ratio $R_i = 2$ and subsequently intercalated with the plasmid DNA.

Size expansion of the interlamellae host from 8.8 Å for LDH to 42 Å in the resulting nanobiohybrid was observed (Figure 12a), indicating stable hybridization of the plasmid DNA formed by ion exchange method, as shown schematically in Figure 13. Powder x-ray diffraction (PXRD) patterns, compositional (Table 6) and electrophoresis studies (Figure 12 c) confirmed the encapsulation episode of the plasmid biomaterial. TEM image shows that the nanobiohybrid crystals are of nanometer size Figure 12 (b).

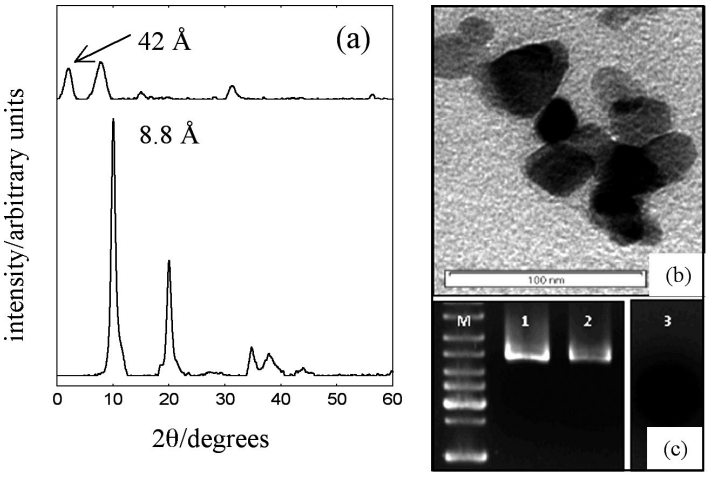


Figure 12 PXRD patterns for LDH and its pEGFP-N2 plasmid-nanobiohybrid (a), TEM image shows nanobiohybrid crystals are in the nanometer range (b) and that the pEGFP-N2 plasmid DNA was successfully recovered from the synthesized nanobiohybrid after treatment with endonucleases (c). Lane 1 shows the plasmid before intercalation, and Lane 2 shows the plasmid recovered from the nanobiohybrid after endonuclease treatment. The LDH host was able to protect the DNA from degradation as compared to naked DNA alone being degraded by the endonucleases in Lane 3. Lane M shows the typical DNA ladder marker (Masarudin *et al.,* 2009).

In order to elucidate the use of the resulting nanobiohybrid as a gene delivery vector, an MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrasodium bromide) assay was performed to determine any cytotoxic effects of the host towards cells. The intercalated pEGFP-N2 anion was later successfully recovered through acidification with HNO_3 after treatment with DNA-degrading enzymes, demonstrating the ability of the LDH host to protect the intercalated biomaterial from degradation.

Table 6 Chemical compositions of Mg/Al-NO_3^- LDH and its nanobiohybrids

Sample	$[Mg(NO_3)_2]$	$[Al(NO_2)_3]$	R_i	R_f	Phase observed
Mg/Al-NO_3^- R_2	0.2 M	0.10	2	2.1	LDH
Mg/Al-NO_3^- R_3	0.2 M	0.07	3	2.9	LDH
Mg/Al-NO_3^- R_4	0.2 M	0.05	4	2.4	LDH
Mg/Al-NO_3^- R_5	0.2 M	0.04	5	5.1	LDH
Nanobiohybrid[a]	0.2 M	0.1	2	2.1	LDH + NB
Nanobiohybrid[b]	0.2 M	0.1	2	2.1	LDH + NB

	elemental content/%		
	C	H	N
Mg/Al-NO_3^- R_2	-	14.27	7.12
Nanobiohybrid[a]	8.43	19.54	6.31
Nanobiohybrid[b]	10.74	20.63	6.10

[a]300 µg/ml pEGFP-N2, [b]300 mg/ml pEGFP-N2, NB = nanobiohybrid, R_i = initial Mg/Al ratio, R_f = final Mg/Al ratio.

Cell transfection studies on Vero3 cells were then performed, where cells transfected with the nanobiohybrid exhibited fluorescence as early as in the 12 h post-treatment but not in cells with the controls; plasmid only or host only (Figure 14 (top)). Cellular transfection of Vero3 cells with MgAl–plasmid DNA and Lipofectamine™–plasmid was found to be comparable for both vectors though the LDH-based vector is more economically viable compared to the commercially available Lipofectamine™ (Figure 14 (bottom)).

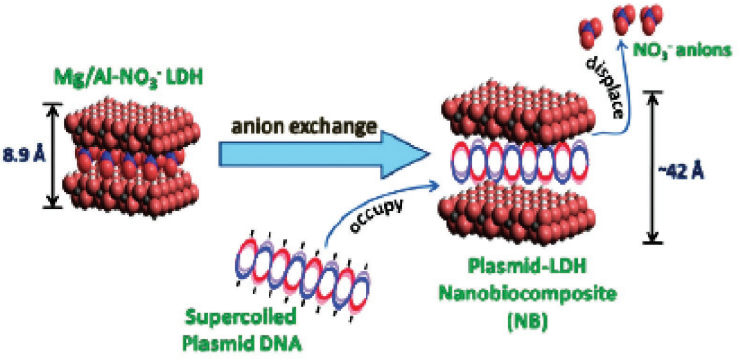
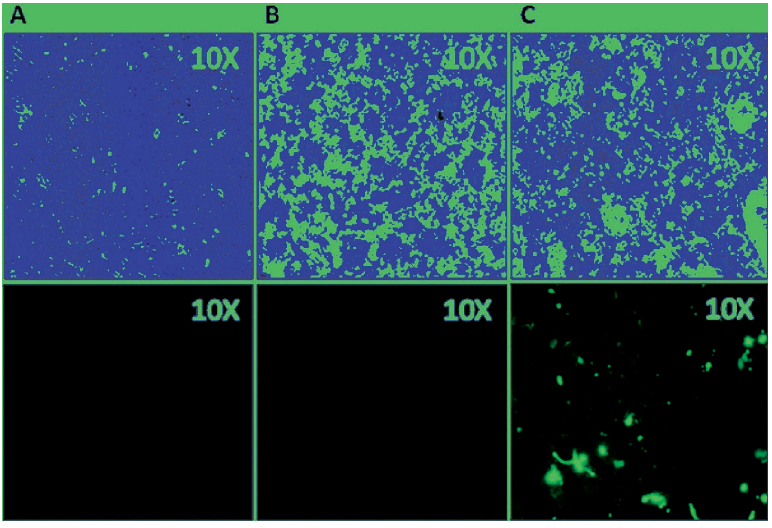


Figure 13 Schematic representation of the formation of Mg-Al–plasmid DNA nanobiohybrid by anion exchange method. A smaller counter anion of the LDH, nitrate in the interlamellae region of LDH was anion exchanged with a bulkier plasmid DNA, leading to an expansion of basal spacing and the formation of the plasmid-LDH nanobiohybrid (Masarudin *et al.,* 2009).



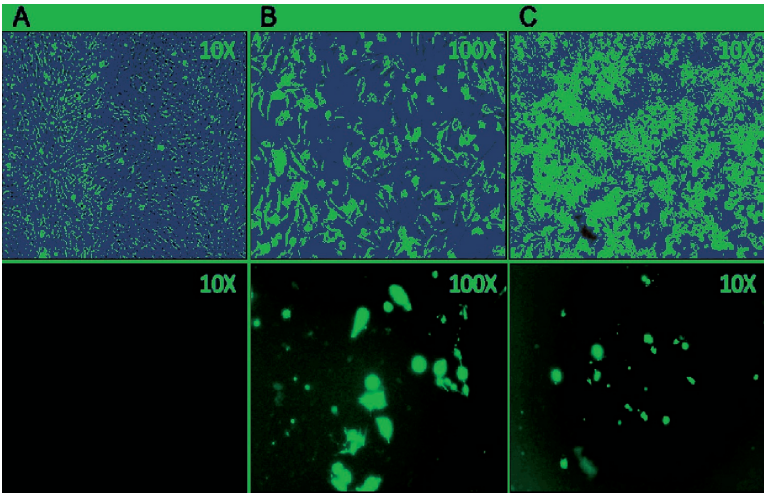


Figure 14 (Page 30): Cellular transfection of Vero3 (African monkey kidney) cells with (A) naked pEGFP-N2 alone, (B) MgAl-NO_3^- and (C) MgAl–plasmid DNA nanobiohybrid samples after 24 h treatment. Fluorescence was observed on cells treated with the nanobiohybrid and not on cells with the controls (plasmid only and host only). In repeated experiments, cells show fluorescence as early as 6 h after treatment (results not shown). (Page 31): Cellular transfection of Vero3 cells with (B) MgAl–plasmid DNA, (B) Lipofectamine™–plasmid DNA samples after 24 h treatment. Fluorescence was observed on cells treated with both the nanobiohybrid and liposome complex but not on (A) control cells (no treatment) (Masarudin *et al.,* 2009).

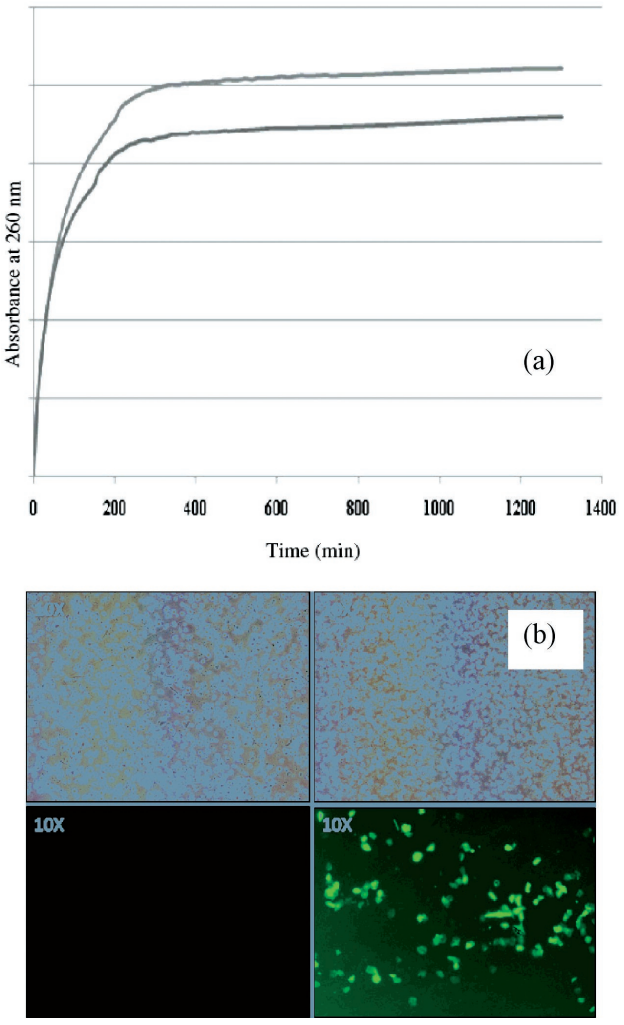


Figure 15 Release of plasmid pEGFP-N2 from inorganic LDH host (a), and cellular transfection of the pEGFP-N2 plasmid into WeHi3b tumor suspension cells. Transfection was successful using the nanobiohybrid (right) compared to naked pEGFP-N2 alone (left). The inset numbers show the microscope magnification used (b) (Masarudin *et al.,* 2009).

Release of plasmid pEGFP-N2 from inorganic LDH host was found to be of controlled manner Figure 15a. Cellular transfection of the pEGFP-N2 plasmid into WeHi3b tumor suspension cells was also carried out (Figure 15b). Transfection was successful using the nanobiohybrid (right) compared to the use of naked pEGFP-N2 alone (left). Transfection success using the nanobiohybrid in difficult-to-transfect cells such as this demonstrates its flexibility and high potential use as a delivery vector. It also showed that the nanobiohybrid can also be used to transfect cells in difficult-to-transfect conditions such as in suspensions.

TRANSFORMATION OF LDH ON HEAT TREATMENT

Heat treatment of LDH at low temperature resulted in removal of adsorbed water or, to some extent, interlayer water. In addition, basal spacing can be also changed slightly (Hussein *et al.,* 1995). On the other hand, upon severe heat treatment the inorganic layered structure of LDH collapsed, and formation of various phases could be observed, depending on the composition as well as type of M^{2+} and M^{3+}. In case of Zn-Cr-LDH, where M^{2+} and M^{3+} is Zn and Cr respectively, heating at 350 ^{O}C or higher resulted in the collapse of the layered structure followed by formation of ZnO as the dominant phase, (Figure 16i).

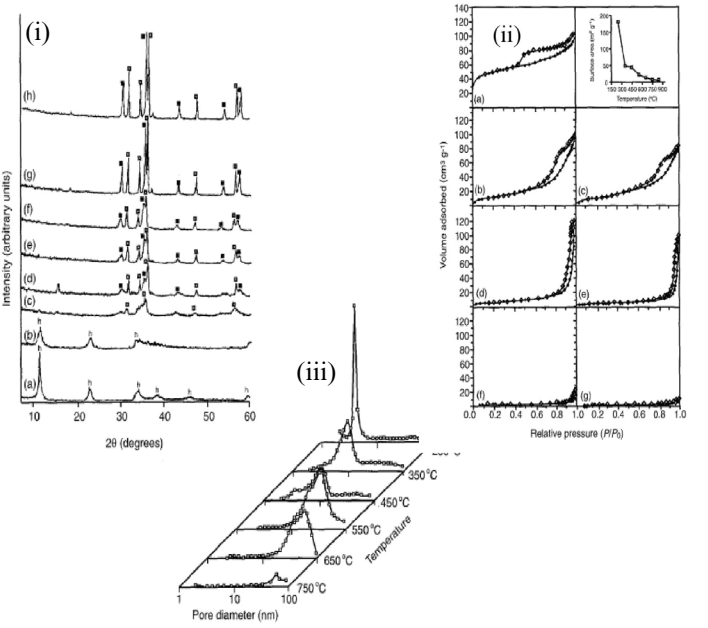


Figure 16 Phase transformation of Zn-Cr-LDH on heating at various temperatures; a = 250, b = 350, c = 450, d = 550, e = 650 and f = 750 ^{o}C (i) effect of temperature on the BET surface area (top right) and the adsorption isotherms (ii) and on pore size distribution (iii) (Hussein *et al.,* 1995).

As a result, surface properties such as morphology and surface area of the products are also changed as shown in Figures 16 and 17. Various morphologies are formed due to different phase transformations and the surface area reduces when the heating temperature increases (Figure 16 (ii) top right). In addition, different types of pores are formed as indicated by the different adsorption isotherms and pore size distribution (Figures 16 (ii) and (iii).

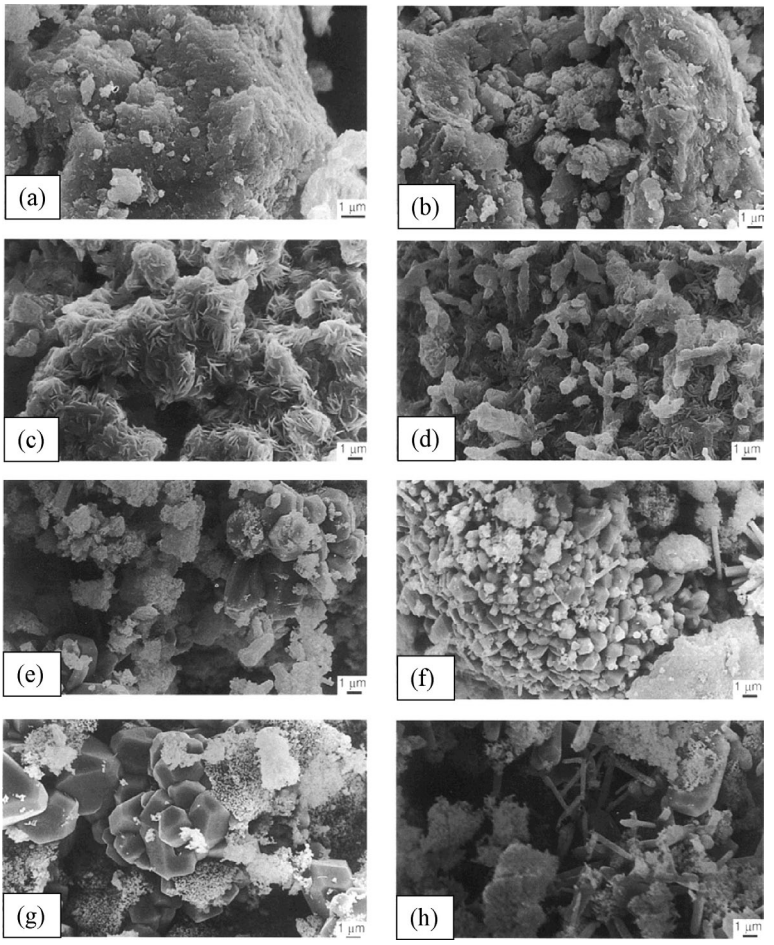


Figure 17 Surface morphology of as-prepared ZnCrCl-LDH (a), and its calcined products, HTLDHs heated at (b) 250, (c) 350, (d) 450, (e) 550, (f) 650, (g) 750 and (h) 850 °C (Hussein *et al.,* 1995).

Due to memory effect property, re-generation of the LDH phase is possible when the heat-treated products are contacted with anions suitable to be used as counter anion in aqueous media. This can also be used as a means to generate new nanohybrid compounds.

High temperature treatment of LDHs, especially those composed of Ni, Fe and Co result in well dispersed nanoparticles of the metal oxides. This opens up the possibility of using such LDHs as the catalyst precursors for CNTs.

LDH as Catalyst Precursor for Preparation of Carbon Nanotubes

The Carbon nanotube, CNT is one of the carbon allotropes known today. Other forms of carbon include diamond, graphite, carbon black and activated carbon. The unique physico-chemical properties of CNTs have attracted intense research, in particular on their synthesis methods and possible up scaling for mass production. The promising properties of CNTs enable them to be used in various fields from composites to sensors and from electronics to medical.

CNTs are chemically stable and have large specific surface area, hollowness and light mass. Their physical properties are expected to be dependent on the size and shape of catalytic nanoparticles which control the growth of CNTs and this would require an efficient and reproducible route for catalyst preparation.

The growth of CNTs by thermal chemical vapour deposition (CVD) has been reported to be relatively simple and reproducible (Edgar, 2005). Various growth models and thermodynamic analysis have been reported for the nucleation and growth of CNTs. In this approach, it is important to prepare an effective catalyst with appropriate size of active metal particles, usually Fe, Co or Ni or their combinations.

As was mentioned earlier, high temperature treatment of LDHs, especially those composed of Ni, Fe and Co result in well dispersed nanoparticles of the metal oxides. This opens up the possibility of using such LDHs as the catalyst precursors for CNTs. CNTs of

multi-walled type, MWNTs, can be prepared over Ni-Al-LDH by the catalytic decomposition of haxene using the CVD technique. High stability of the Ni nanoparticles prepared *in situ* by the decomposition of this catalyst allows better dispersion of the active catalytic sites and is hence expected to result in the high yield growth of MWNTs.

Ni-Al-LDH with carbonate as the counter anion was synthesised and the resulting basal spacing of 7.6 Å was observed for the Ni-Al-LDHs synthesised at pH 7.5 (Figure 18). The material was subsequently calcined under atmospheric environment at 800 ^{o}C, resulting in the collapse of the layered structure. The reason for choosing calcination temperature of 800 ^{o}C is because the same temperature will be used for the formation of the CNTs. In addition, the LDHs without calcination at 800 ^{o}C will also be used for comparison. As a result of the calcination, new phases, namely NiO, Ni and Al can be observed as indicated in the figure.

The resulting calcined product of Ni-Al-LDH at 800 ^{o}C was used as a substrate and catalyst for the formation of CNTs. The XRD pattern shows a new peak, which could be attributed to the CNTs (002), can be observed, together with Al and Ni (Figure 18c). Similar XRD pattern was observed when Ni-Al-LDH was used directly without any pre-treatment (Figure 18d), but the NiO phase can still be observed. This shows that NiO was not fully transformed to Ni when pre-calcination was not done before the formation process of the CNTs on the substrate takes place, as the time is shorter taking only 1 hour to reach 800 ^{o}C, compared to the 5 h in the pre-calcined materials.

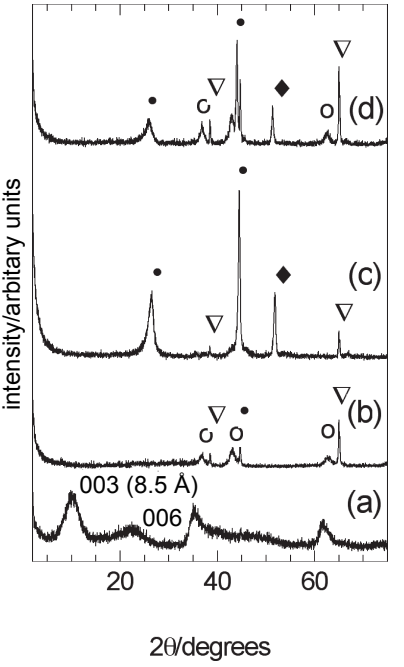


Figure 18 PXRD diffraction patterns for the Ni-Al-LDH (a), its calcined product at 800 °C for 5 h under flowing air (b), the resulting CNTs growth on sample b (c), and the CNTs growth on Sample a directly without any pre-treatment as in sample b (d), Al (♦), CNT/carbon 002 (•), Ni (∇), NiO (o).

Curled multi-walled carbon nanotubes were obtained when haxene was flowed through the calcined LDH without prior reduction. This was done without any catalyst added, showing that the formation of nickel nanoparticles was actually accomplished and act as catalyst. Similar formation of MWNTs was also observed when various LDHs, FeCoNiAl-LDH, CoNiAl-LDH, FeNiAl-LDH and NiAl-LDH were heat-treated at 800 °C for 5 h under atmospheric conditions and used as catalysts without prior reduction (Figures 19c-f).

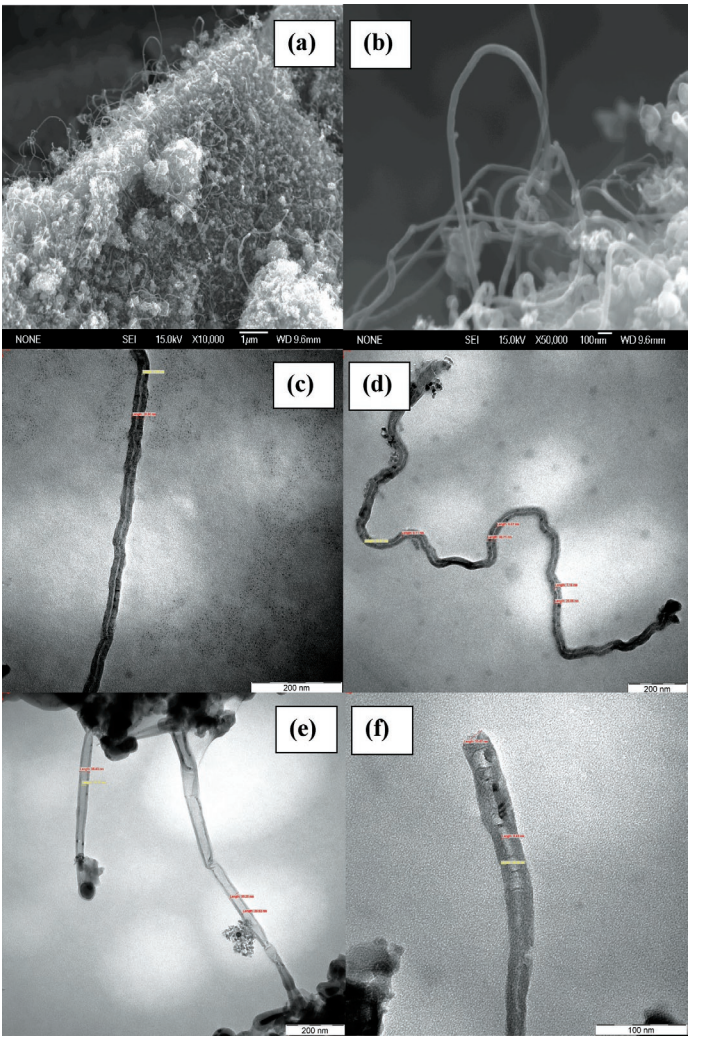


Figure 19 Curled multi-walled carbon nanotubes observed by FESEM on the calcined LDH without prior reduction (a), higher magnification of the MWNTs (b). TEM image of multi-walled carbon nanotubes obtained using various types of LDHs as catalysts by heating them at 800 °C without prior reduction, FeCoNiAl-LDH (c), CoNiAl-LDH (d), FeNiAl-LDH (e), and NiAl-LDH (f).

Effect of Substrate on Physical Properties of Carbon Nanotubes

Formation of CNTs using various substrates shows that CNTs of various shapes, sizes and orientations can be obtained. Preparation was accomplished using the chemical vapour deposition (CVD) technique with ferrocene as catalyst and haxene as carbon source. For all the substrates used, multi-walled CNTs are the dominant product.

When zirconia was used as a substrate, curled MWNTs are observed with various sizes and random orientation (Figs. 20a -c). Substrate of o-sialon gives curled MWNTs with slightly lower aspect ratio than the one growing on zirconia (Figure 20d).

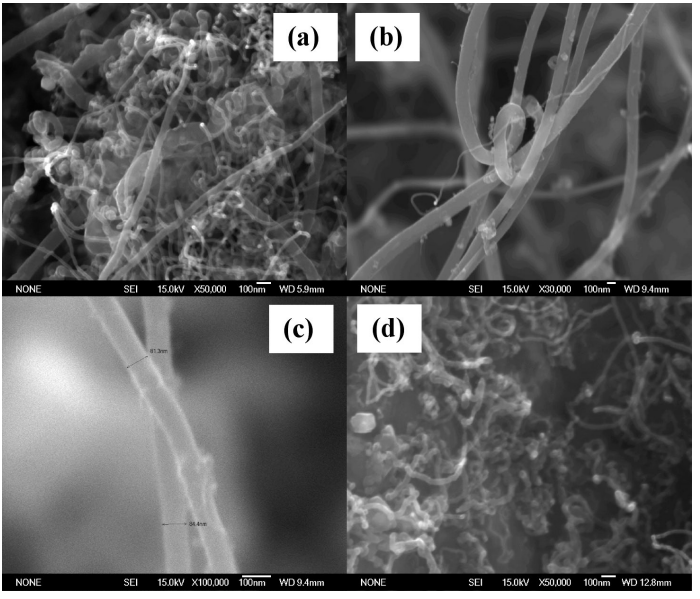


Figure 20 Curled MWNTs formed on a zirconia substrate (a), close up of some MWNTs showing various diameters (b), part of the straight MWNTs with diameter of around 80 nm (c), MWNTs formed on an o-sialon substrate (d).

Vertically aligned MWCTs can be obtained when the catalyst, ferrocene, was flowed at a constant flow rate by haxene/nitrogen mixture and deposited on a wall of quartz glass as shown in Figure 21a. Good distribution of catalysts of nanosize together with "tip growth" mechanism is believed to give vertically aligned MWNTs a "carpet-like" structure of uniform size and similar aspect ratio. TEM images show that the CNTs are of MWCNT type (Figures 21 b-c) composed of various layers, and higher magnification shows that the MWCNTs have about 23 layers (Figure 20d).

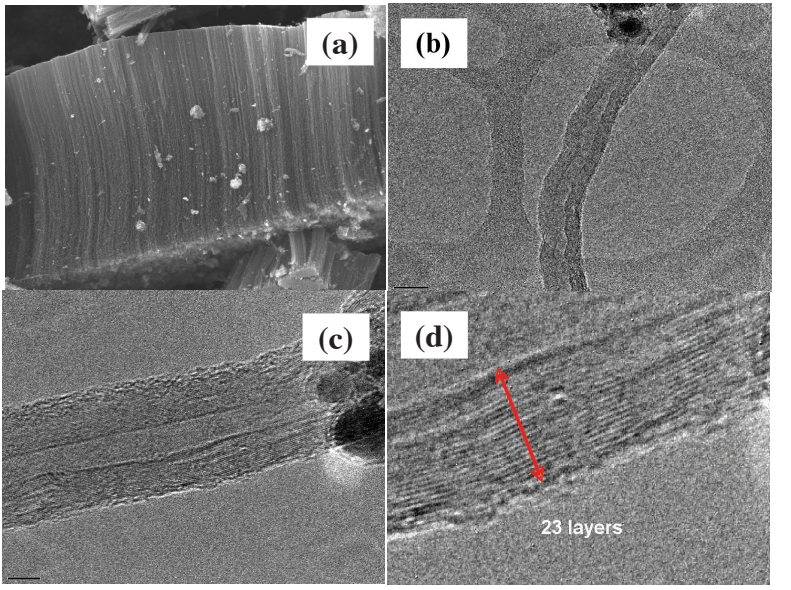


Figure 21 Vertically aligned MWCTs formed on a quartz surface with "carpet-like" structure (a), TEM image of CNT showing the walls of MWCNTs composed of various layers (b) and (c) and higher magnification showing that the MWCNTs are composed of about 23 layers (d).

ZINC OXIDE NANOPARTICLES

Lately, ZnO of various sizes and shapes have been widely used in numerous technological applications, especially in the area of biosensors in medical diagnostics (Zhang *et al.,* 2006) catalysts (Akyol *et al.,* 2004), gas sensors (Baruwati *et al.,* 2006) and electronic nanodevices (Singh *et al.,* 2007). This is because zinc oxide, a wurtzite-type, II-IV semiconductor with a direct band gap of 3.37 eV at room temperature has unique properties that meet the requirements of various applications.

ZnO with desired morphology and dimension for enhancing nanostructure order is another key issue in this area of research. Template-hydrothermal method is one of many approaches that can be used to address this issue. The technique requires the presence of a template, so that the desired nanomaterials can be formed on it and the resulting nanostructure is directed by the rich shape and morphology of the template. It is essential that the template is stable towards the reaction conditions during the synthesis, (Huczko, 2006). Good control and stable morphology during final template removal is another major factor that has to be considered.

The use of highly rigid-structure and biofunctionality-based activities of microbial cells as biotemplating agent meet the requirements discussed earlier. This is because the interactions between metal-microorganism have already been studied and employed in biotechnological applications (Vijayaraghavan and Yun, 2008). However, the formation of metal oxide nanoparticles with specific shapes by using biotemplating agents has not been fully explored compared to the bio-assisted synthesis of metal nanoparticles.

BACTERIA AS BIOTEMPLATES

Synthesis of ZnO using biotemplate coupled with the hydrothermal method is another technique that can be used to prepare ZnO and other metal oxides with desired morphology and dimensions for enhancing nanostructures. Templating agents, chemical or biological materials, are usually added during the aging stage so that the metal oxide can grow and form on them. Otherwise the metal cations can be added during the growing stage of the bacteria, so that the biochemistry processes involved can be used as a means for the formation of various nanostructures. The resulting materials are then calcined at predetermined temperature under atmospheric condition, and the template will be burnt out resulting in formation of metal oxide with enhanced nanostructures.

Bacteria are among the good candidates for use as biotemplates for this purpose. *Acetobacter xylinum* and *Baccilus cereus* were selected for this study. Formation of zinc oxide nanostructures was found to be possible.

Acetobacter xylinum

Acetobacter xylinum is a carbohydrate producing bacterium and it is widely used for formation of *nata de coco* (NDC) (Figure 22 a), a jelly-like foodstuff. This type of bacterium secretes crystallized cellulose microfibrils (Figures 22b-d). The bacterium is used as a construction worker on the nanoscale by depositing aligned ribbons of cellulose on a templated surface, a nanobiotechnology process. *A. xylinum* secretes cellulose microfibrils from one end, driving itself forward with aligned tracts of glucan chains less than 1 nm apart. On this surface, the bacteria deposited around 100 nm wide cellulose ribbons in the direction of the template tracts at a rate of 4.5 micron per minute at 24 ^{o}C (Tetsuo, 2005).

ZnO was prepared through hydrolysis of zinc acetate in the presence of the bacteria *A. xylinum* at various amounts, as a shape-directing agent or biotemplating agent, (Table 6). The bacterium was used in the formation of *nata de coco* to mimic the resulting shape desired (Hussein *et al.,* 2005). The XRD pattern of the as-synthesized ZnO shows that a relatively pure phase can be obtained (Figure 23). The amount of templating agent added plays a role in determining the physicochemical properties of the resulting synthesized material, especially the specific surface area (Table 6).

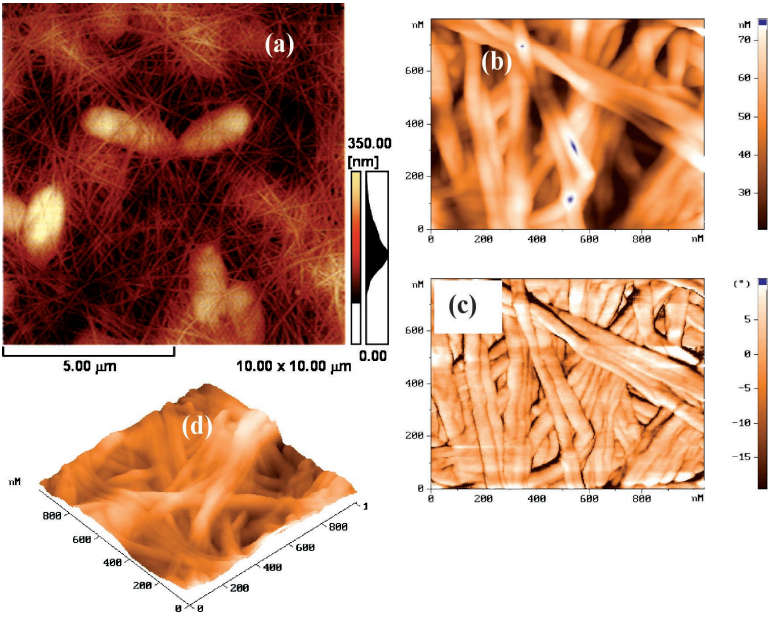


Figure 22 SPM image of *nata de coco* comprising the bacteria, *Acetobacter xylenium* (nodule-like) and celulose (thread-like) (a), and detailed structure of the cellulose microfibrils secreted by the bacteria (b-d).

Table 6 ZnO synthesised using various amounts of *A. xylenium* as biotemplating agent and their BET specific surface area.

Sample	Amount of NDC (gL^{-1})	BET surface area (m^2/g)
ZnO	0.00	1.0
ZnO-a	0.67	7.1
ZnO-b	1.67	12.8
ZnO-c	2.67	13.6

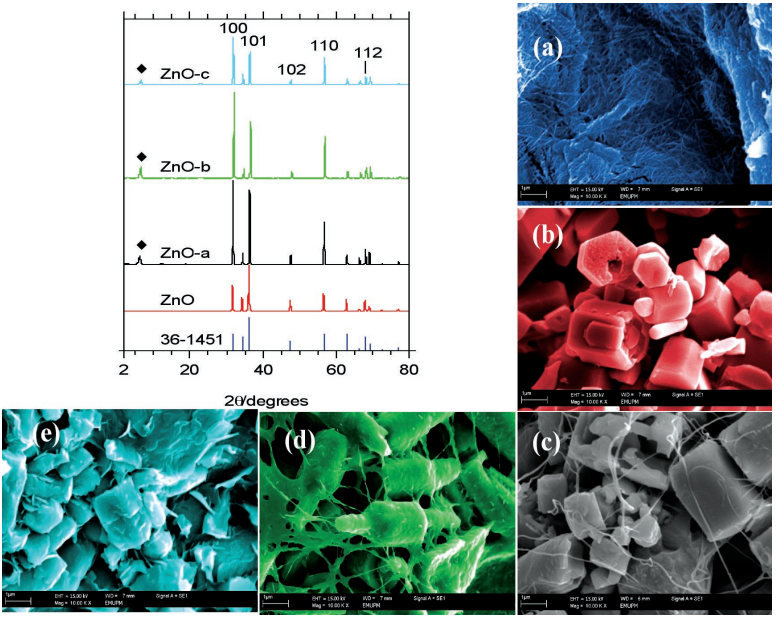


Figure 23 PXRD patterns for ZnO synthesized without *nata de coco* (ZnO) and in the presence of *nata de coco* at various amounts; 0.67 g/L (ZnO-a), 1.67 g/L (ZnO-b), and 2.67 g/L (ZnO-c). SEM images of dried *nata de coco* showing cellulose with thread like (a), ZnO (b) ZnO-a (c), ZnO-b (d), ZnO-c (e) (Hussein *et al.,* 2005).

Figure 23 shows SEM images of the ZnO synthesized at different amounts of *nata de coco*, 0.00–2.67 g/L. As shown in the figure, ZnO synthesized in the absence of *nata de coco* resulted in a mixture of various sizes and shapes. However, hexagonal is the dominant shape. On the other hand, the presence of *nata de coco* resulted in the presence of thread-like structure together with various shapes of ZnO. In addition, a bottle-like shape can be clearly observed. The thread-like and bottle-like shapes (Figure 23 d) can be correlated to the known structure of *nata de coco* which are due to the cellulose and the bacteria (Figure 22 a), respectively.

Bacillus cereus

B. cereus is an endemic, gram-positive rod-shaped bacteria (Figure 24 a) that causes food poisoning. It is responsible for food borne illnesses, causing severe nausea, vomiting and diarrhea. This occurs due to survival of the bacterial endospores when food is improperly cooked, allowing the endospores to germinate. Bacterial growth results in production of enterotoxin and ingestion leads to diarrhea and emetic (vomiting) syndrome.

Nanostructured zinc oxide with raspberry- and plate-like structures was synthesised using *B. cereus* as a biotemplating agent by making use of the biochemistry processes of the bacteria (Hussein *et al.,* 2008). Zn-templated *B. cereus* was obtained by addition of zinc cation into the culture, and the bacteria allowed to grow for 48 h. At this stage no ZnO phase can be observed (Figure 24bi). However, PXRD patterns of Zn-templated *Bacillus cereus,* after calcination at 500 °C shows ZnO pure phase, similar to the one synthesised by direct method. No ZnO phase can be observed when calcination was not done (Figure 24bi). This shows that the bacteria disintegrated on heating and at the same time phase transformation to ZnO occurred.

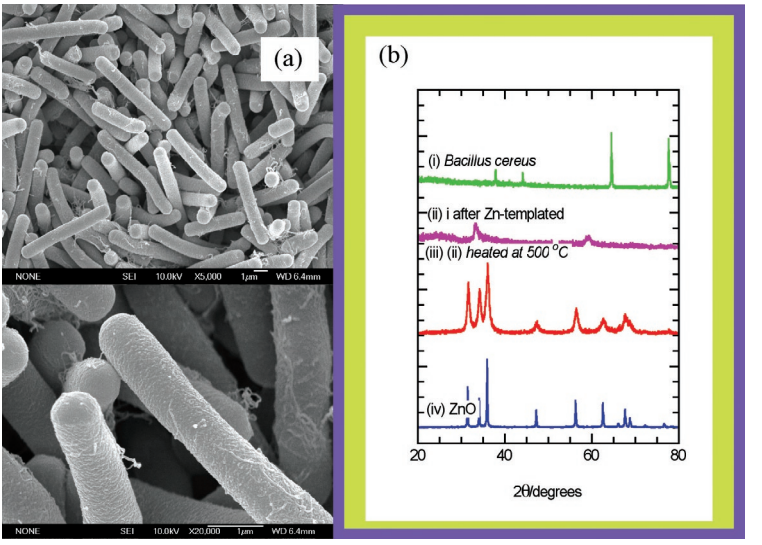


Figure 24 Rod-like shape of *B. cereus* at low and high magnification (a) and PXRD patterns for *B. cereus* (b); (i), Zn-templated *B. cereus* (ii) Zn-templated *B. cereus* after calcination at 500 °C (iii) and ZnO obtained by direct synthesis (iv).

The mechanism of nanostructured ZnO formation is proposed based on basic combinations of cellular biochemistry processes including metal ions transportation, activation of metal-binding sites, intracellular metal ions accumulation and metal cation oxidation on heating. The description of this mechanism is proposed as shown schematiclly in Figure 25. The shape of the ZnO nanostructures formed would depend on the organelles or membrane on which they are nucleated and templated.

Zn^{2+} ions enter the cells through the plasma membrane by specific and unspecific transports, as well as by receptor-mediated endocytosis (Campbell and Reece 2002, Chen *et al.,* 2008) through 'open gate' transporter. The accumulated Zn^{2+} act as precursor for ZnO nanoparticles formation on heating as proposed in Figure 25.

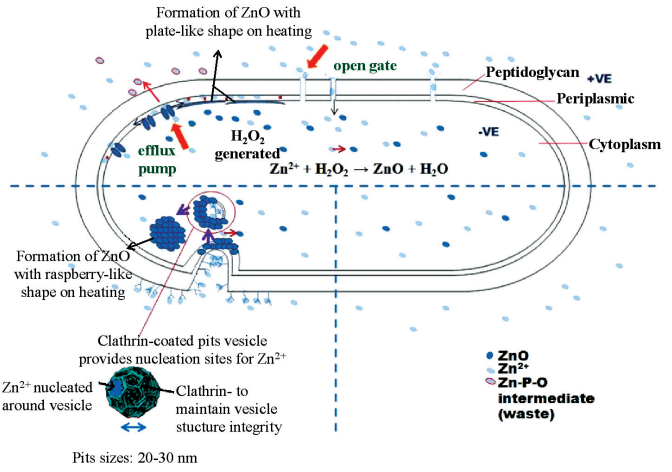


Figure 25 Proposed mechanisms for the formation of ZnO with raspberry- and plate-like nanostructures.

The nucleation and growth of metal cation will take place at the bacterial organelles in the cells. Zn^{2+} will complex with the Zn-binding receptor and acumulate on the organelles. The complexation between uncomplexed metal divalent cations with deprotonated organic acid functional group at Zn-binding receptor accumulate the Zn^{2+} ions at the outer bacterial surfaces, leading these sites to provide further nucleation sites. Once these sites are transported into the cells by receptor-mediated endocytosis in the form of a sphere-like shaped vesicle known as the clathrin-coated pit, they act as geochemically reactive solids (Gilbert *et al.,* 1997). The surface of this Zn^{2+}-rich vesicle will provide nucleation surfaces and the Zn^{2+} ion will be accumulated and templated within each pore that is formed as a result of the arrangement of clathrin protein around this vesicle. Upon calcination, the organic matter of the organelles will be burnt out, resulting in Zn^{2+}-clathrin coated pit vesicles being oxidized and becoming ZnO with raspberry-like shape.

At the same time, cytoplasmic membrane and periplasm will initiate the nucleation sites for Zn^{2+} at the surface of plasma membrane inside the cells. On heating, the final shape of the ZnO formed at these sites are plate-like shaped. This shape is also formed in the surrounding inner membrane plasma surface of the bacterium.

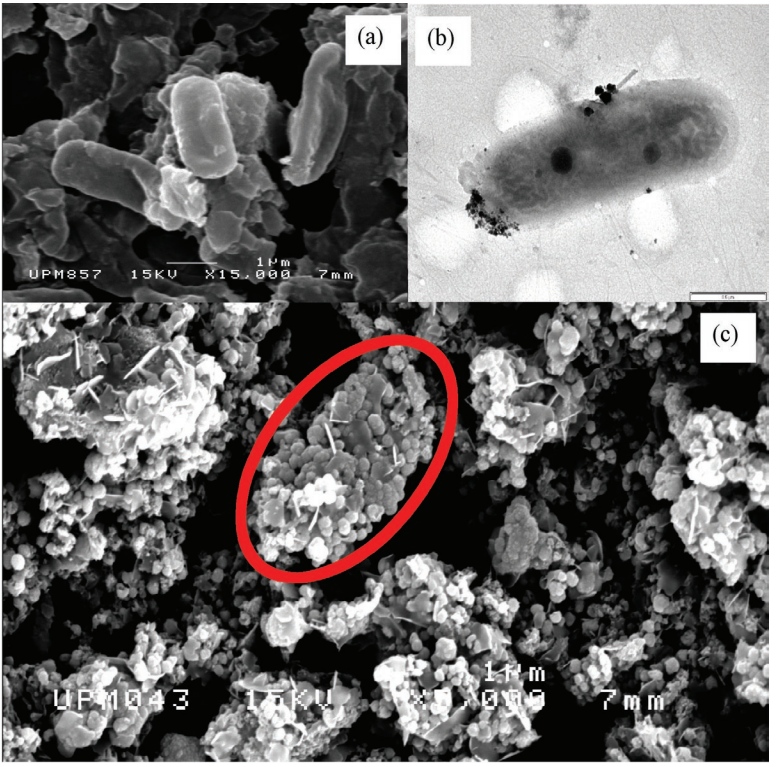


Figure 26 FESEM micrograph of Zn-templated *B. cereus* (a), TEM micrograph of Zn-templated *B. cereus* (b), Zn-templated *B. cereus* after calcination at 500 °C for 4 h showing disintegration of the outer layers of the bacteria, peptidoglycan and periplasmic membranes, producing nodules and plate-like structures agglomerated together in a few groups. A group in a circle is produced by a bacterium.

FESEM micrograph of Zn-templated *B. cereus* (Figure 26 a) shows distorted rod-like structure and TEM micrograph of Zn-templated *B. cereus* (Figure 26b) shows similar rod-like structure with the presence of granules of various sizes (dark-coloured). FESEM micrograph of Zn-templated *B. cereus* after heating at 500 ^{o}C for 2 h shows nodules and plate-like structures agglomerated together in a few groups. The presence of agglomerated groups is due to the disintegration of the outer layers of the bacteria on heating, in which each group is due to a single bacterium. The granules and plate-like structures are due to the formation of ZnO, as a result of oxidation of Zn^{2+}-rich organelles and plasma membranes, respectively.

Figure 27a shows the FESEM micrograph of the as-synthesized ZnO nanostructures after biotemplate removal by calcination for 4 h at 500 ^{o}C under atmospheric condition which indicate the presence of granules and plate-like shapes. At higher magnifications, the granules show raspberry–like shape (Figure 27b) and the raspberry-like shape is actually composed of nodules with sizes of around 20-30 nm and the thickness of the plate-like shape is around 25 nm (Figure 27c).

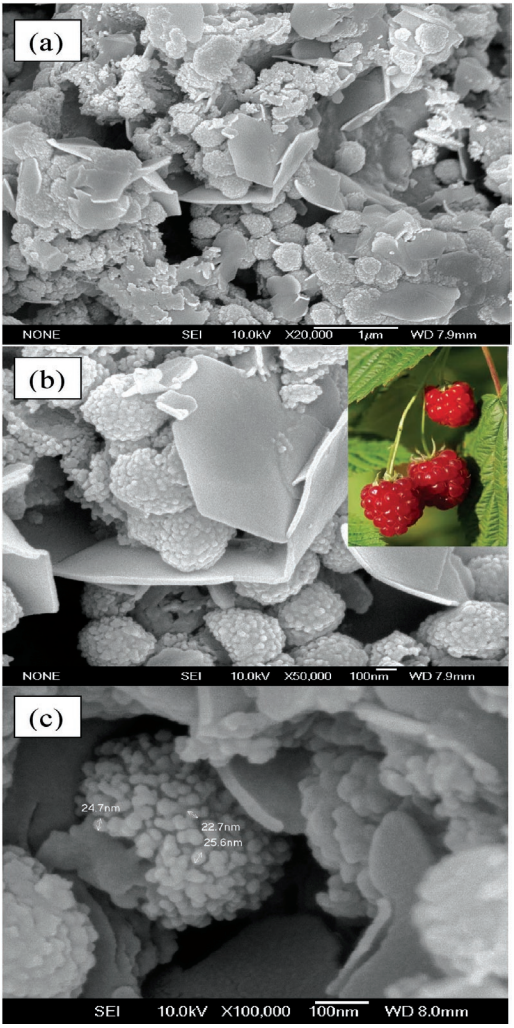


Figure 27 FESEM micrograph of Zn-templated *B. cereus* after heating at 500 °C for 4 h showing granules and plate-like structures (a), at higher magnification, the granules show raspberry-like structure with raspberry fruits (inset) (b), and the raspberry-like shape composed of nodules of around 20-30 nm diameter and the plate-like shapes of around 25 nm thick (c).

Polyol-hydrothermal method

ZnO nanoparticles can also be synthesised using the polyol-hydrothermal method. Polyols, namely ethylene glycol (EG), diethylene glycol (DEG) and triethylene glycol (TEG) were used and polyol-dependent particle size distributions of the ZnO nanoparticles generated (Figure 28). The average particles size of 39, 37 and 45 nm, measured using photon cross correlation spectroscopy (PCCS), was obtained when EG, DEG and TEG were used, respectively. The particle size distribution shows single-modal with skewed distributions which are polyol-type dependent, and the order of the broadness of the distribution can be given as EG > DEG > TEG (Figure 28a). Such an order is similar to the size order of the polyols, and we believe that the size of the polyol controls the size and distribution of the nanoparticles formed.

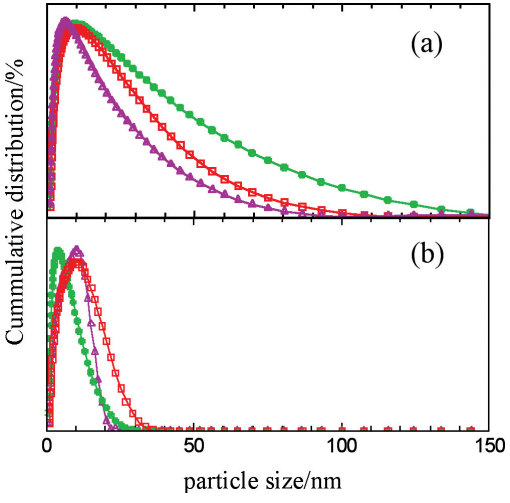


Figure 28 Particle size distribution of zinc oxide nanoparticles synthesised using polyol-hydrothermal method in the presence of EG (●), DEG (□) and TEG (Δ) before (a) and after being (b) heat-treated at 500 °C for 4 h under atmospheric condition.

On heat treatment at 500 °C under atmospheric condition for 4 hours, the skewed particle size distribution which extended to around 150 nm, significantly reduced to around only 40 nm, and at the same time the average particle size also reduced to 19, 25 and 15 nm, for EG, DEG and TEG, respectively (Figure 28b).

PXRD patterns show that both ZnO nanoparticles are of pure phase with hexagonal-wurtzite structure (JCPDS No. 01-075-0576) (Figure 29). The presence of broad and sharp absorption bands at around 440 cm^{-1} in the FTIR spectrum (not shown) read just the y axis (expand it) supported the existence of the ZnO phase.

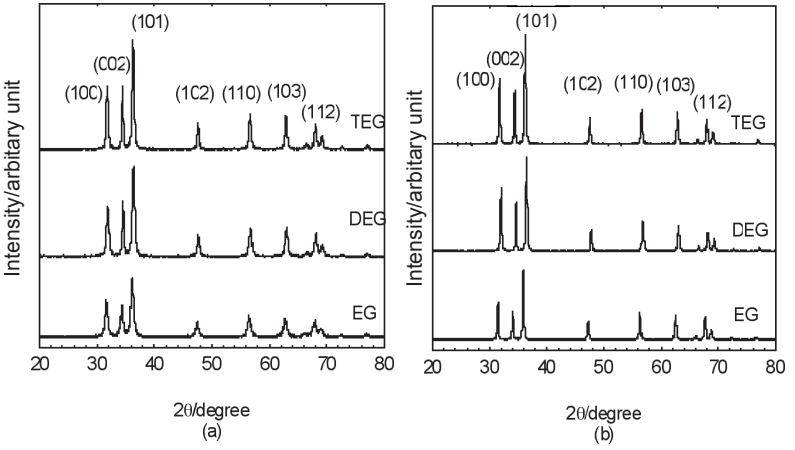


Figure 29 PXRD patterns of zinc oxide nanoparticles synthesised using the polyol-hydrothermal method using EG, DEG and TEG (a) and their calcined products (b).

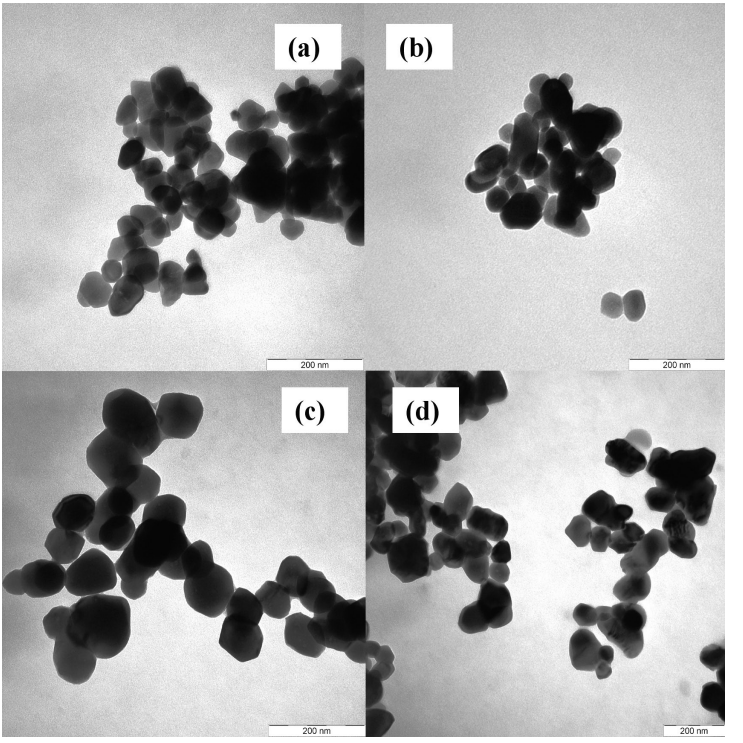


Figure 30 TEM images of ZnO nanoparticles synthesized by polyol-hydrothermal method before being heat-treated using EG (a) and TEG (b), and their products after heat-treatment at 500 ^{o}C for 4 hours of a (c) and b (d).

The TEM image (Figure 30) shows poor correlation between the sizes observed with the particle size distributions obtained earlier. The presence of bigger particles shown by TEM shows that smaller particles are actually aggregated together forming larger secondary and tertiary agglomerations as depicted by the FESEM study, shown in Figure 31. This shows that the particle size distributions measured by PCCS technique is mainly due to the primary and, to some extent, to the secondary particles size.

Although the extent of size distributions of the particles decreased tremendously, no significant morphology transformation can be clearly observed when the ZnO nanoparticles were heat-treated at 500 ^{o}C for 4 h (Figure 32).

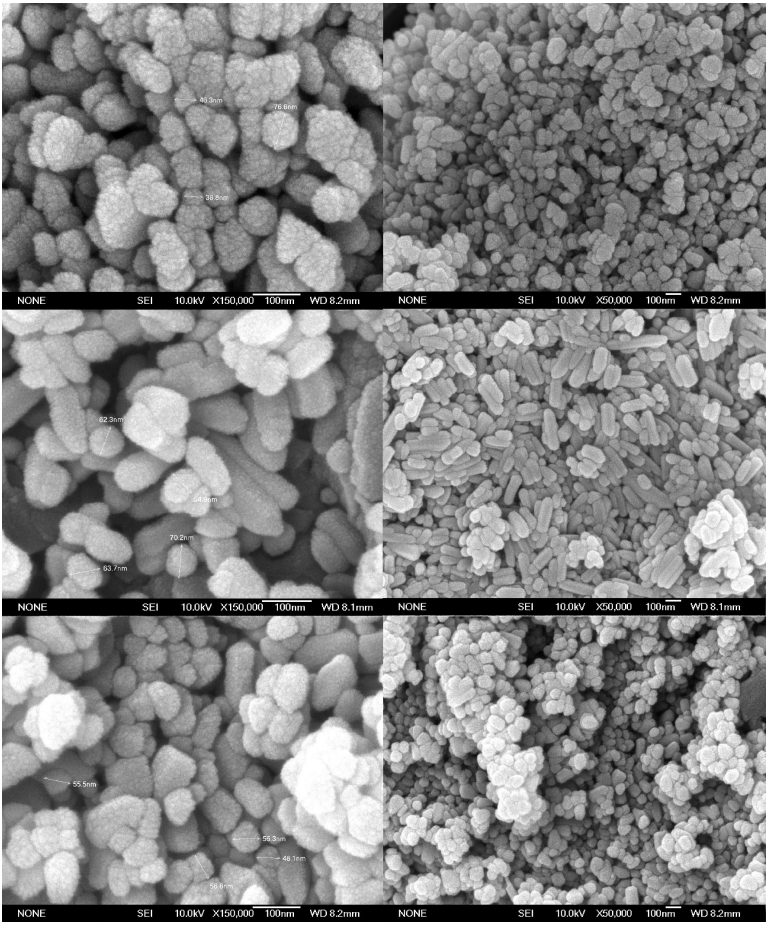


Figure 31 FESEM micrographs of ZnO nanoparticles synthesized by polyol-hydrothermal method using EG, DEG and TEG (from top to bottom), at higher magnification (left) and lower magnification (right).

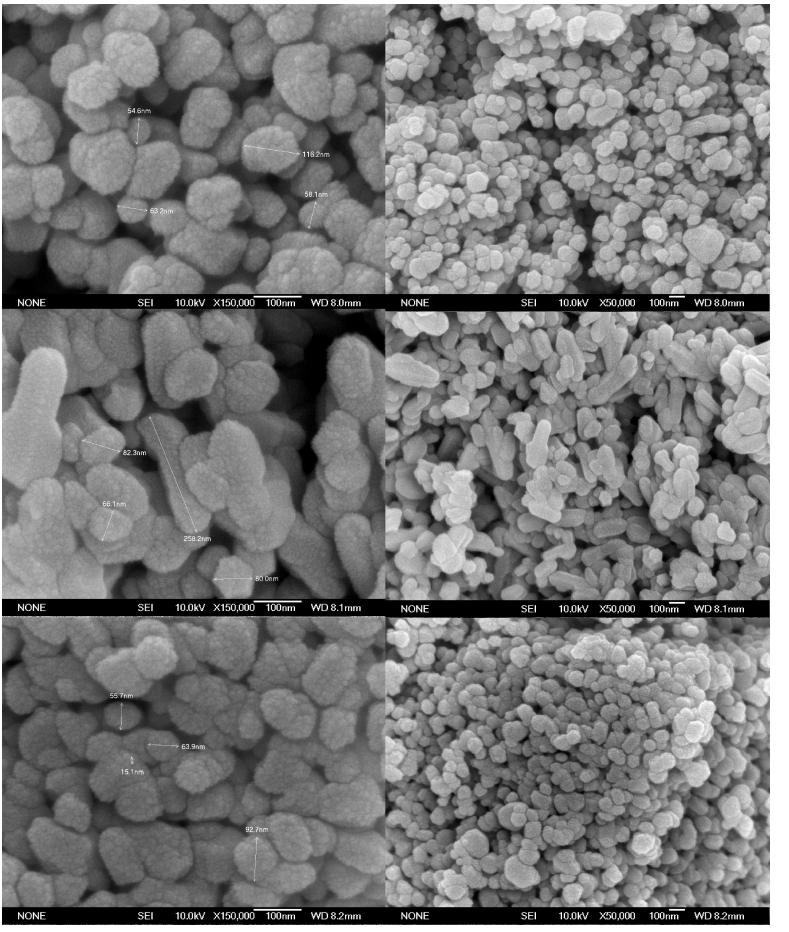


Figure 32 FESEM micrographs of ZnO nanoparticles synthesized by polyol-hydrothermal method using EG, DEG and TEG (from top to bottom) heated at 500 °C for 4 h under atmospheric condition at higher magnification (left) and lower magnification (right).

As shown in the FESEM micrographs, ZnO nanoparticles showed cylinder-like shape when DEG was used during the synthesis, compared to a more rounded one when EG and TEG

were used (Figure 31), and no significant size transformation can be clearly observed on heating (Figure 32).

ZnO with Carnation-Like Nanostructures

ZnO nanoparticles have flexibility to form different nanostructures and the formation process is due to the effect of small particles which are arranged and agglomerated together. As a result, formation of different nanostructures can be observed.

Uniform, larger monodispersed semi-spherical particles with nanometer sized petal thickness are formed, believed to be initially aggregated from the primary particles. The particles adhere to each other with very specific orientation and adherence occurs due to weak Van der Walls forces, leading to the carnation-like shape shown in Figure 33.

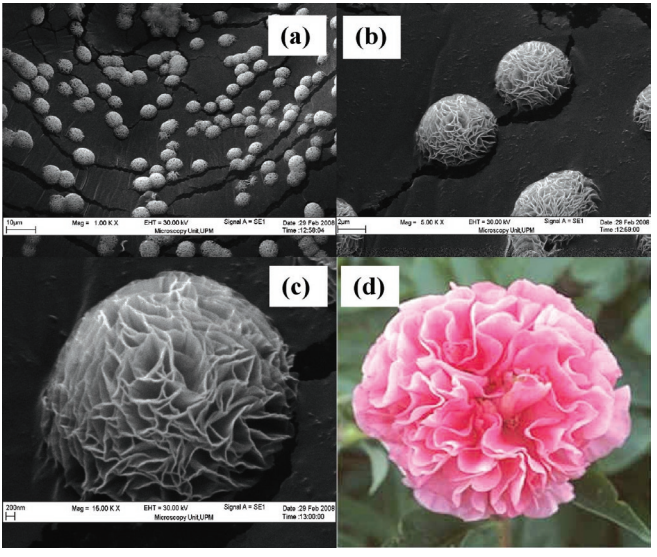


Figure 33 Zinc oxide synthesised using the DEG-hydrothermal method showing carnation-like nanostructure shape at various magnifications (a-c) and a carnation flower (d).

CONCLUSION

Efforts have been made to learn to control materials' properties at the atomic scale using the wet chemistry method, especially in the design and formation of layered inorganic hybrid materials containing beneficial agents such as organic or biological materials to synthesise various host-guest type of nanohybrids with sizes of 1-100 nm, as well as processing and characterization of nanostructured and nanoparticles produced by self-assembly methods. This is because these types of materials exhibit physico-chemical properties which are different from their counterparts and the properties can be tailor-made by choosing the correct combination of host-guest species.

Work on nanomaterials especially the layered 2D type is particularly important in the synthesis of a new generation of nanohybrid agrochemicals for controlled release formulation, useful for environmental protection and as ecomaterials. On the other hand, research on novel nanohybrid materials containing drugs or biomaterials has a very promising future and good commercial potential for functional materials, especially for drug and gene delivery with controlled release capability and nanobiotechnology with improved functional capability. The host-guest type of materials can be used to store beneficial agents in the nanolayered interlamellae, transport it to the target and ultimately controlled release.

LDHs composed of iron, cobalt or nickel, or their binary or ternary combinations, calcined at high temperature are suitable for use as catalyst precursors in carbon nanotube preparation. This is due to the better dispersion of the metal nanoparticles achieved, which is essential for the formation of multiwalled carbon nanotubes. Using biomaterial such as bacteria as the biotemplate coupled with the hydrothermal method to generate new nanostructured

materials, opens up avenues for new methods of synthesis by the green chemistry route.

This work provides understanding of the important fundamental considerations necessary for the design and formation of various novel nanohybrids and other nanomaterials. It opens avenues for a new class of nanomaterials with physical and chemical properties that can be tailor made.

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BIOGRAPHY

Mohd Zobir bin Hussein is a Professor of Chemistry, Faculty of Science, Universiti Putra Malaysia. He was born in Muar Johor and his early primary and secondary education was in Muar. He accomplished his upper secondary school at Sekolah Alam Shah, Kuala Lumpur. He graduated with B. Sc. (Hons) in Chemistry from UKM and Ph.D in Chemical Spectroscopy from the University of Reading, England.

He together with his colleagues has devoted his career at UPM to studying nanomaterials and nanobiomaterials. He and his research group dedicated their work on the organic-inorganic nanohybrids and nanobiohybrids containing beneficial agents such as drugs, organic or biological materials to synthesise host-guest nanohybrids, as well as processing and characterization of nanoparticles, carbon nanotubes and nanostructured materials.

As nanotechnology is multidiscipline in nature, collaboration with other research groups of various disciplines is essential. Collaborations with researchers from various research areas in biotechnology and biomolecular sciences, chemistry, enzyme technology, biology and physics enable him and his group to move forward exploring new research frontiers, in particular nanobiotechnology. This enable his group's research findings to be published in various journals in the area of nanoscience and nanotechnology such as Nanotechnology, Journal of Nanoscience and Nanotechnology, Current Nanoscience, Journal of Nanoparticle Research and other local and international journals.

The work was supported by more than 60 postgradute students under him as the chairman or member of supervisory committee, which resulted in 130 publications in international/national journals and another 200 papers published in international and national proceedings, abstracts, reports, and 3 patents.

In recognition of his contributions to the University, UPM has awarded him with Vice Chancellor Fellowship 2006 (Excellent in Research), UPM Excellent Service Awards and Excellent Service Certificates. The Vice Chancellor Fellowship enabled him to carry out research on nanoparticles at the Center for Strategic Nano-Fabrication, University of Western Australia. He was also awarded the Overseas Advanced Research Fellowships Scheme for research attachment at the Macdiarmid Institute of Advanced Materials and Nanotechnology, New Zealand, the Marie Curie European Union Fellowship for research attachment at the University of Southhampton, England and the Debye Institute for Nanomaterials Science, University of Utrecht, the Netherland, the British High Commissioner's Chevening Award for research attachment at the University of Southampton, England, the Public Service Department of Malaysia/Asian Development Bank Fund Training Award for research attachment at the Material Research Laboratory, Pennsylvania State University, USA. He has also been awarded many prizes and medals at International, National and the University level competitions for his group's research works.

Zobir is also an active referee for some international/national journals. He has also served as the External Assessor for B. Ed. Chemistry Programme, UPSI. He is a former Head of Advanced Materials Laboratory, ITMA UPM, Coordinator of Postgraduate Studies, the then Faculty of Science and Environmental Studies, UPM.

Zobir is currently a Fellow and Life Member of Malaysian Solid State Science (MASS), Associate of Malaysian Institute of Chemistry (AMIC), Member of Malaysian Analytical Chemistry Society (ANALIS), Member of Material Research Society (USA) and member and committee member of Malaysian Nanotechnology Association (MNA).

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First of all, I would like to express my gratitude to all my collegues and collaborators in the Department of Chemistry, Biology and Physics, Faculty of Science, Department of Virology Faculty of Biotechnology and Biomolecular Sciences, Advanced Materials and Nanotechnology Laboratory, Institute of Advanced Technology (ITMA), Institute of Bioscience (IBS), Centre of Excellence in Catalysis Science and Technology, Faculty of Science, Faculty of Agriculture, all the postgraduate and undergraduate students working in my research group as well as other individuals for being together with me in this knowledge seeking endeavour.

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Australia), Prof. J.L. Spencer (MacDiarmid Institute of Advanced Materials and Nanotechnology, Victoria University of Wellington, New Zealand), Prof. M.T. Weller (University of Southampton, England), Prof. A. Meijerink (Debye Institute for Nanomaterials Science, University of Utretch, the Netherland), Dr P.B. Malla and Prof. S. Komarneni (Material Research Laboratory, Pennsylvania State University, USA) and Dr J.M. Hollas (University of Reading). All of them have inspired me in developing my skill and passion in research and making it as a wonderful amazing journey.

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LIST OF INAUGURAL LECTURES

1. Prof. Dr. Sulaiman M. Yassin
   The Challenge to Communication Research in Extension
   22 July 1989

2. Prof. Ir. Abang Abdullah Abang Ali
   Indigenous Materials and Technology for Low Cost Housing
   30 August 1990

3. Prof. Dr. Abdul Rahman Abdul Razak
   Plant Parasitic Nematodes, Lesser Known Pests of Agricultural Crops
   30 January 1993

4. Prof. Dr. Mohamed Suleiman
   Numerical Solution of Ordinary Differential Equations: A Historical Perspective
   11 December 1993

5. Prof. Dr. Mohd. Ariff Hussein
   Changing Roles of Agricultural Economics
   5 March 1994

6. Prof. Dr. Mohd. Ismail Ahmad
   Marketing Management: Prospects and Challenges for Agriculture
   6 April 1994

7. Prof. Dr. Mohamed Mahyuddin Mohd. Dahan
   The Changing Demand for Livestock Products
   20 April 1994

8. Prof. Dr. Ruth Kiew
   Plant Taxonomy, Biodiversity and Conservation
   11 May 1994

9. Prof. Ir. Dr. Mohd. Zohadie Bardaie
   Engineering Technological Developments Propelling Agriculture into the 21st Century
   28 May 1994

10. Prof. Dr. Shamsuddin Jusop
    Rock, Mineral and Soil
    18 June 1994

11. Prof. Dr. Abdul Salam Abdullah
Natural Toxicants Affecting Animal Health and Production
29 June 1994

12. Prof. Dr. Mohd. Yusof Hussein
Pest Control: A Challenge in Applied Ecology
9 July 1994

13. Prof. Dr. Kapt. Mohd. Ibrahim Haji Mohamed
Managing Challenges in Fisheries Development through Science and Technology
23 July 1994

14. Prof. Dr. Hj. Amat Juhari Moain
Sejarah Keagungan Bahasa Melayu
6 Ogos 1994

15. Prof. Dr. Law Ah Theem
Oil Pollution in the Malaysian Seas
24 September 1994

16. Prof. Dr. Md. Nordin Hj. Lajis
Fine Chemicals from Biological Resources: The Wealth from Nature
21 January 1995

17. Prof. Dr. Sheikh Omar Abdul Rahman
Health, Disease and Death in Creatures Great and Small
25 February 1995

18. Prof. Dr. Mohamed Shariff Mohamed Din
Fish Health: An Odyssey through the Asia - Pacific Region
25 March 1995

19. Prof. Dr. Tengku Azmi Tengku Ibrahim
Chromosome Distribution and Production Performance of Water Buffaloes
6 May 1995

20. Prof. Dr. Abdul Hamid Mahmood
Bahasa Melayu sebagai Bahasa Ilmu- Cabaran dan Harapan
10 Jun 1995

21. Prof. Dr. Rahim Md. Sail
Extension Education for Industrialising Malaysia: Trends, Priorities and Emerging Issues
22 July 1995

22. Prof. Dr. Nik Muhammad Nik Abd. Majid
The Diminishing Tropical Rain Forest: Causes, Symptoms and Cure
19 August 1995

23. Prof. Dr. Ang Kok Jee
The Evolution of an Environmentally Friendly Hatchery Technology for Udang Galah, the King of Freshwater Prawns and a Glimpse into the Future of Aquaculture in the 21st Century
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24. Prof. Dr. Sharifuddin Haji Abdul Hamid
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25. Prof. Dr. Yu Swee Yean
Fish Processing and Preservation: Recent Advances and Future Directions
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26. Prof. Dr. Rosli Mohamad
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27. Prof. Dr. Mohamed Ismail Abdul Karim
Microbial Fermentation and Utilization of Agricultural Bioresources and Wastes in Malaysia
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28. Prof. Dr. Wan Sulaiman Wan Harun
Soil Physics: From Glass Beads to Precision Agriculture
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29. Prof. Dr. Abdul Aziz Abdul Rahman
Sustained Growth and Sustainable Development: Is there a Trade-Off 1 or Malaysia
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30. Prof. Dr. Chew Tek Ann
Sharecropping in Perfectly Competitive Markets: A Contradiction in Terms
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31. Prof. Dr. Mohd. Yusuf Sulaiman
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32. Prof. Dr. Abu Bakar Salleh
Enzyme Technology: The Basis for Biotechnological Development
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33. Prof. Dr. Kamel Ariffin Mohd. Atan
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34. Prof. Dr. Ho Yin Wan
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35. Prof. Dr. Tan Soon Guan
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36. Prof. Dr. Nazaruddin Mohd. Jali
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37. Prof. Dr. Abdul Rani Bahaman
Leptospirosis-A Model for Epidemiology, Diagnosis and Control of Infectious Diseases
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38. Prof. Dr. Marziah Mahmood
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42. Prof. Dr. Wong Kai Choo
Advancing the Fruit Industry in Malaysia: A Need to Shift Research Emphasis
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43. Prof. Dr. Aini Ideris
Avian Respiratory and Immunosuppressive Diseases- A Fatal Attraction
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44. Prof. Dr. Sariah Meon
Biological Control of Plant Pathogens: Harnessing the Richness of Microbial Diversity
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45. Prof. Dr. Azizah Hashim
The Endomycorrhiza: A Futile Investment?
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47. Prof. Dr. Muhamad Awang
Do We Have Enough Clean Air to Breathe?
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48. Prof. Dr. Lee Chnoong Kheng
Green Environment, Clean Power
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49. Prof. Dr. Mohd. Ghazali Mohayidin
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50. Prof. Dr. Fatimah Mohd. Arshad
Analisis Pemasaran Pertanian di Malaysia: Keperluan Agenda Pembaharuan
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51. Prof. Dr. Nik Mustapha R. Abdullah
Fisheries Co-Management: An Institutional Innovation Towards Sustainable Fisheries Industry
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52. Prof. Dr. Gulam Rusul Rahmat Ali
Food Safety: Perspectives and Challenges
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Nutrient Management Strategies for Sustainable Crop Production in Acid Soils: The Role of Research Using Isotopes
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54. Prof. Dr. Maisom Abdullah
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55. Prof. Dr. Wan Omar Abdullah
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56. Prof. Dr. Syed Tajuddin Syed Hassan
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58. Prof. Dr. Ahmad Zubaidi Baharumshah
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59. Prof. Dr. Shaik Md. Noor Alam S.M. Hussain
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61. Prof. Dr. Annuar Md. Nassir
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64. Prof. Dr. Han Chun Kwong
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65. Prof. Dr. Mawardi Rahmani
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88. Prof. Dr. Mohd Arif Syed
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125. Prof. Dr. Azni Idris
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